Cut-400616-74-P25701

ISSN 0006-8950

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Brain

A JOURNAL OF NEUROLOGY

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VOLUME 114 · PART IA · FEBRUARY 1991

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Brain (ISSN 0006-8950) is published bi-monthly by Oxford University Press, Southfield Road, Eynsham, Oxford OX8 1JJ, England. Annual subscription price in USA $195.00 including air speed delivery Second class postage paid at Jamaica, NY 11431

USA POSTMASTERS. Send address changes to *Brain*, Publications Expediting, Inc, 200 Meacham Avenue, Elmont, NY 11003. Air freight and Mailing in the USA by Publications Expediting, Inc, 200 Meacham Avenue, Elmont, NY 11003.

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Continued on inside back cover

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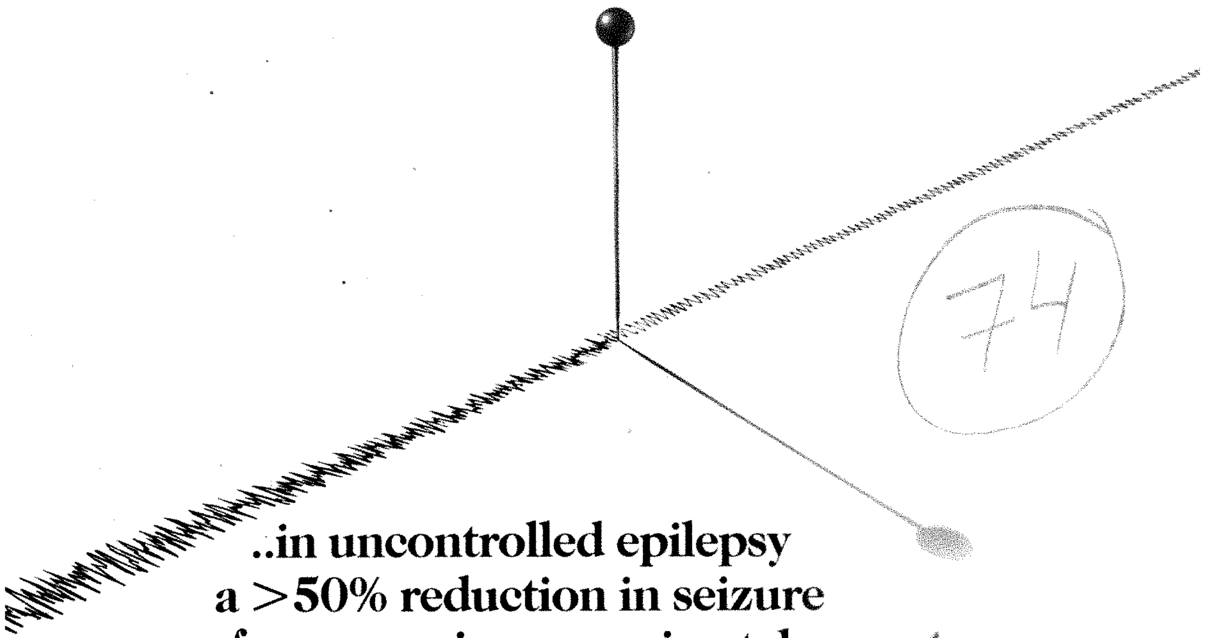

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EDITORIAL ANNOUNCEMENT

As is also being experienced by other journals of clinical neurology, *BRAIN* continues to receive increasing numbers of papers of high quality. In order to reduce the interval between acceptance of a paper and its publication, Volume 114 Part I of *BRAIN* has been issued in two parts, IA and IB. For the remainder of 1991, the usual single bimonthly issues will be maintained. It needs to be emphasized again that although *BRAIN* is prepared to accept longer papers if appropriate, shorter papers, if documenting complete studies are also acceptable. Preliminary reports are not acceptable, neither are papers that are unduly verbose or loquacious.

From 1 January 1991, the editorship of *BRAIN* has changed. New papers should now be submitted to Professor W. I. McDonald, Editor of *BRAIN*, Institute of Neurology, National Hospital for Neurology and Neurosurgery, Queen Square, London WC1N 3BG, UK. Instructions for the submission of papers will be found on the inside of the front cover.

P. K. Thomas

Brain (1991), **114**, 1–11

THE NECK-EYE REFLEX IN PATIENTS WITH REDUCED VESTIBULAR AND OPTOKINETIC FUNCTION

by ADOLFO M. BRONSTEIN, STUART MOSSMAN *and*
LINDA M. LUXON

(From the Medical Research Council Human Movement and Balance Unit, Institute of Neurology, and the Department of Neuro-Otology, National Hospital for Neurology and Neurosurgery, Queen Square, London, UK)

SUMMARY

It is accepted that the neck-eye loop (cervico-ocular reflex, COR) is enhanced following loss of vestibular function and that this helps to restore gaze stability during head movements. In this paper we address the question of which structures and/or mechanisms may participate in such plastic enhancement by investigating the COR in 2 patients with absent vestibular function and reduced smooth pursuit-optokinetic eye movements (SP-OKN). The patients had multisystem atrophy involving the vestibular system and the cerebellum. The COR (elicited by angular motion of the trunk relative to the fixed head and angular motion of the head relative to the fixed trunk) was not enhanced in these 2 patients when compared with normal subjects, in contrast to previous findings in a group of patients with absent vestibular function alone. Measurements of slow phase eye movement velocity during SP-OKN stimuli and during combined COR-OKN stimulation (head oscillation relative to the stationary trunk in the light) showed identical values in these two conditions, which indicates that the neck-eye loop did not contribute to gaze stability during head movements. The absence of plastic enhancement of the COR in these patients may be secondary to interruption of SP-OKN pathways at various possible sites and/or to involvement of the vestibulocerebellum, which is known to mediate adaptive plasticity in the vestibulo-ocular reflex.

INTRODUCTION

The vestibulo-ocular reflex (VOR) maintains retinal image stability during head movements by generating slow phase compensatory eye movements in an opposite direction and at an equal velocity to that of head motion. The gain of this reflex in the light (gain is the ratio between peak velocity of the slow phase eye movement and peak head velocity) is close to unity. It has been argued that, in order to keep the gain so finely tuned throughout life, the VOR must undergo continuous recalibration (Robinson, 1976) and this view has been supported by the demonstration of so-called 'plastic changes' occurring in the vestibular and oculomotor systems. Probably the best known example of such adaptive changes is the modification of the VOR by wearing telescopic lenses, or optically reversing prism goggles, which significantly affect the gain and even the direction of the VOR (Gonshor and Melvill Jones, 1976*a*, *b*; Lisberger, 1984). It has been shown that removal of the vestibulocerebellum or inferior olive abolishes this form of plasticity (Robinson, 1976; Haddad *et al.*, 1980; Lisberger *et al.*, 1984; *see* Miles and Lisberger, 1981; Ito, 1982; and Lisberger, 1988, for reviews).

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Plasticity in the oculomotor system has also been demonstrated by the adaptive changes occurring in the neck-eye loop following loss of vestibular function. In the normal monkey, eye movements elicited by cervical stimulation (cervico-ocular reflex, COR) are negligible but, after bilateral labyrinthectomy, gradually become more powerful and generate significant compensatory eye movements during normal head motion (Dichgans *et al.*, 1973). Although this finding has subsequently been confirmed in other species (Baker *et al.*, 1982) and in man (Kasai and Zee, 1978; Barnes, 1979; Takahashi *et al.*, 1981, 1989; Bles *et al.*, 1984; Chambers *et al.*, 1985; Bronstein and Hood, 1986; Halmagyi and Curthoys, 1987; Leigh *et al.*, 1987), there have been no animal or clinical experiments aimed at identifying which mechanisms or neural structures mediate these adaptive changes (Dichgans, 1975). In this paper, we report the findings of experiments on cervically and visually induced eye movements in 2 patients in whom absent vestibular function was accompanied by reduced smooth pursuit-optokinetic (SP-OKN) eye movements due to concomitant cerebellar involvement. If a failure of enhancement of the COR was identified in such patients, it could be postulated that the cerebellum, or pursuit-optokinetic mechanisms, play a part in mediating the plastic changes occurring in the COR after vestibular loss.

MATERIAL AND METHODS

Case reports

Case 1

This 51-yr-old man presented with a 9 yr history of progressive unsteadiness in the dark and episodes of vertigo, some of them positionally induced. For 2 yrs, he had also noted dysarthria, a hoarse voice and extreme fatigue. Examination revealed a markedly ataxic gait with a tendency to fall to the right. There were mild cerebellar signs in all four limbs, but no pyramidal or sensory signs or postural hypotension. Tendon reflexes and plantar responses were normal. A combined assessment of eye movements clinically and with d c. electro-oculography (EOG) showed absent convergence; frequent square waves, first degree gaze evoked nystagmus to right and left and rebound nystagmus. Spontaneous downbeat nystagmus with eyes 30° right and left was noted, which also appeared on clinical examination in the primary position, on performing the Nylen-Hallpike positional test. Pursuit was markedly deranged and optokinetic responses revealed a reduction in slow phase velocity bilaterally. Saccades were dysmetric horizontally and vertically. Compensatory eye movements during passive oscillation of the patient's head, whilst voluntary visual fixation was maintained (doll's head), were markedly broken up ('cogwheel'). The patient had no nystagmic response to the following vestibular stimuli in the dark. rotational velocity steps of ±40 and 80 deg/s, sinusoidal rotation at 0.2−0.4 Hz with a peak velocity at 40 deg/s and caloric irrigation with water at 20° C for 1 min. Audiometric tests were normal. An EMG showed evidence of an axonal sensorimotor neuropathy, with absent sensory action potentials and reduced interference pattern, but normal conduction velocities. CT and MRI scans showed cerebellar and brainstem atrophy. The clinical picture suggested a sporadic form of olivopontocerebellar atrophy or a multisystem atrophy.

Case 2

This 54-yr-old man had impotence for 10 yrs and a 6 yr history of small, illegible handwriting, frequency of micturition, slurred speech and difficulty in walking. Examination at that time showed parkinsonism partially responsive to L-Dopa. He developed progressive unsteadiness, with falls and urinary incontinence, and more latterly dysphagia and noctural stridor, with excessive snoring. Current clinical examination showed evidence of cerebellar, extrapyramidal and pyramidal features There was a scanning dysarthria with quiet monotonous speech and impassive facies with decreased blink rate. The patient walked with a festinating stooped gait with postural instability. There was right-sided spasticity, hyperreflexia and an extensor plantar response with bilateral ataxia of the limbs and decreased joint position sensation in the feet at the metatarsophalangeal joints. There was no postural hypotension. Combined clinical and EOG examination of eye movements revealed markedly broken pursuit, first degree nystagmus to the right and left, normal saccades and poor convergence. Compensatory eye movements during doll's head manoeuvre were markedly broken up. Vestibular responses in the dark to velocity steps of ±40 and 80 deg/s, sinusoidal rotation (0.2–0.4 Hz, 40 deg/s peak velocity) and standard caloric irrigation at 30 and 40° C were absent. Anal spincter EMG showed denervation and reinnervation consistent with neuronal loss. Urodynamic studies showed an underactive detrusor without outflow obstruction. CT and MRI scans showed slight dilatation of the fourth ventricle with a marked reduction in size of the vermis, pons and medulla. It was concluded that the patient had a multisystem atrophy.

Methods

The experimental strategy with these 2 patients consisted of (1) assessing the COR without interference from visually elicited eye movements (in darkness) and (2) determining the origin of the compensatory eye movements elicited by head motion in the light.

Experiments in the dark

These included horizontal angular motion of the trunk, while keeping the head earth-fixed (trunk motion) and motion of the head around the static trunk (head motion).

Trunk motion. The subjects sat on a rotatory chair which, for safety, was moved by hand by the experimenter. The head was fixed to a sturdy frame attached to the wall by means of a head clamp and either a bite plate or a chin rest. Stimuli consisted of ramp and sinusoidal displacements of the chair. Ramp displacements were randomized in direction with an amplitude of ±10–50° at peak velocities ranging between 10 and 20 deg/s. Before each discrete trunk movement a target light-mounted in line with primary gaze was briefly presented in order to correct for any residual eye deviation which may have been induced by the preceding stimulus. The subjects were encouraged to relax the neck and to look at the target light when it was switched on. Additional trials followed in which the subjects were instructed to look straight ahead during the trunk movement, as if the target light was on all the time ('imaginary target'). The real target light was presented between stimuli in the usual manner. Sinsusoidal stimuli at 0.3 Hz were applied by keeping motion of the chair synchronized to a tone at an approximate amplitude of ±25°C. Trunk motion was transduced by a velocity and a position analogue output from the rotating chair.

Head motion. A helmet connected to a low torque potentiometer and firmly attached to the subject's head was used to monitor head angular displacement. Sinusoidal stimuli at an amplitude of approximately ±25° synchronized with a tone at 0.3 Hz were used. The subject was encouraged to relax the neck and let the experimenter move the head by hand; head and helmet were firmly held in order to minimize any head slippage during motion. Only Case 1 was studied, in 3 different modes: without additional instruction, during mental arithmetic and during imaginary fixation of the earth-fixed target light.

Experiments in the light

These included sinusoidal motion of a visual target (SP-OKN), of the whole body or of the head at 0.3 Hz with a peak velocity of ±40 deg/s (peak amplitude ±20°).

Target motion. The subjects sat inside a full-field black and white striped motorized drum (radius 75 cm), to which an additional 1.5° textured target was attached, and were instructed to follow the target.

Head and trunk ('en bloc') rotation. The subjects sat on a motorized rotating chair (Contraves Goerz Inc.) with the head rigidly fixed by means of an occipital support and a binaural clamp. During oscillation they were requested to keep fixation on a textured target attached to the wall of the room, 140 cm away from the eye.

Head on trunk rotation. The head of the subjects was passively rotated by hand by the experimenter while the patients fixated a textured target on the wall of the room. Head position was monitored with the helmet as described above. The required frequency was achieved by following a tone and the required amplitude by verbal feed-back from experimenters monitoring the signals on an oscilloscope and on the ink recorder.

Eye movements were recorded with bitemporal d.c. EOG with a bandwidth of 70 Hz and displayed on paper charts, together with chair, helmet and OKN drum signals, by means of an ink-jet recorder (Mingograph). Recordings were analysed by hand and expressed as gain (ratio between peak velocity of the slow phase compensatory eye movement and peak velocity of the trunk, head or visual target). Where appropriate, the results were compared with those of a group of 13 normal subjects and 12 patients with absent vestibular function reported by us in recent publications (Bronstein and Hood, 1986, 1987).

RESULTS

Experiments in the dark

Trunk motion

Fig. 1 shows raw eye movement recordings during ramp displacement of the trunk in Case 1, with absent vestibular function and reduced pursuit (right) and, for comparison, in a patient with absent vestibular function, due to gentamicin ototoxicity, but otherwise normal neurological examination (left). Under no instruction, the patient with absent vestibular function alone shows a saw-tooth pattern of nystagmus with the quick

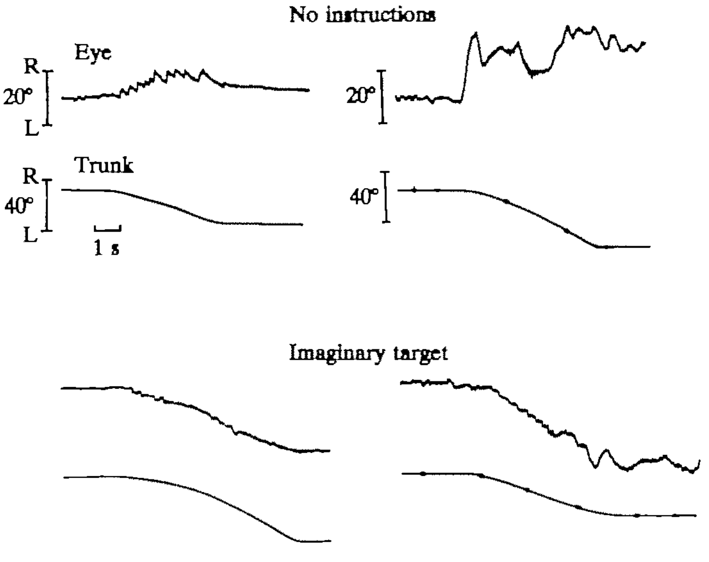

FIG 1. COR responses, elicited by angular ramp displacement of the trunk, whilst the head remains earth-fixed in the dark, in a patient with absent vestibular function alone (*left*) and in a patient with absent vestibular function and deranged pursuit-OKN movements due to cerebellar degeneration (*right*). In the top traces the patients had no specific visuomotor instruction; note the well configurated nystagmic pattern in the patient on the left and its absence in the patient on the right. In the bottom traces the patients had to keep fixation on an imaginary earth-fixed light. The patient on the left suppressed the quick components of the nystagmus so that only the slow phase compensatory eye movement, in the direction opposite to the relative head displacement is retained. In the patient on the right, due to the absence of a meaningful slow phase COR, the compensatory eye deviation in the direction opposite to the relative displacement of the head is mainly achieved by small saccades with a staircase appearance.

component beating in the direction opposite to trunk displacement (i.e., in the same direction as the *relative* displacement of the head upon the trunk) and well configurated slow components in the same direction as trunk motion (i.e., in the opposite direction of the *relative* motion of the head upon the trunk). In contrast, in the 2 patients with combined vestibular and cerebellar degeneration the eye movements present during trunk motion either could not easily be defined as slow or fast (fig. 1), or slow phase eye movements were of very slow velocity, direction changing and not directly related to the stimulus. These various eye movements elicited by trunk motion in Cases 1 and 2 were identical to the COR responses reported in normal subjects (Barnes and Forbat, 1979; Bronstein and Hood, 1986); a regular saw-tooth nystagmic pattern was never found. Attempts to measure slow phase eye velocity were unsuccessful due to the weakness and variability of the response.

In Cases 1 and 2 the predominant overall deviation of the eyes within the orbit was in the direction of the *relative* head movement and was frequently achieved by a large saccade at the beginning of neck torsion as illustrated in fig. 1.

During imaginary fixation (fig. 1, bottom) both in the patient with peripheral vestibular failure alone (left) and in Case 1 with cerebellar and vestibular failure (right), the eyes deviated in the direction opposite to the relative head motion. This is often referred to as the compensatory direction in that, under real head motion, such eye movements would tend to stabilize gaze in space. However, whilst the patient with peripheral vestibular failure alone achieved this mainly by suppressing the quick components of the nystagmus so that the eyes deviated smoothly, Case 1 achieved this by a rapid succession of small saccades. In Case 2, imaginary fixation did not induce a consistent change of the eye movement response.

Fig. 2 shows records during sinusoidal trunk motion stimuli from Case 1 (bottom)

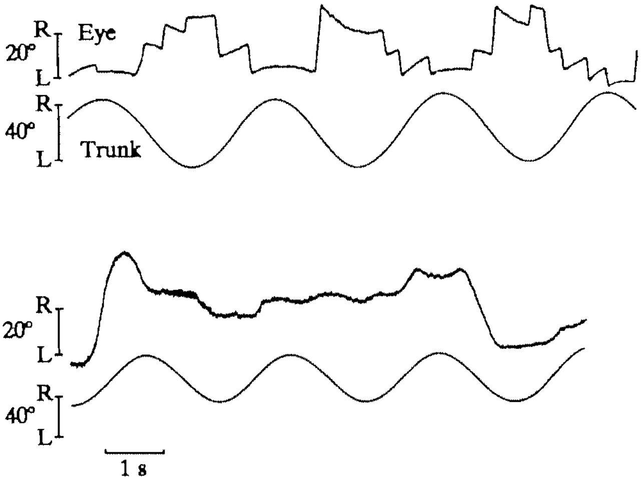

FIG. 2. COR responses elicited by sinusoidal trunk oscillation whilst the head remains earth-fixed The patient with absent vestibular function alone (*top*) shows a regular pattern of nystagmus with the sinusoidally modulated slow phase eye movement in the direction opposite to that of the *relative* displacement of the head. In the patient with absent vestibular function and deranged pursuit-OKN movements due to cerebellar degeneration (*bottom*) no specific pattern of response can be identified.

and, for comparison, from a patient with absent vestibular function, but otherwise normal neurological examination (top), as a sequel of meningitis and aminoglycoside antibiotic treatment. The patient with absent vestibular function alone showed a clear sinusoidal modulation of the nystagmic pattern, the slow phase being consistently directed in the same direction as trunk movement (i.e., in the opposite direction of *relative* head motion). Both patients with defective pursuit and absent vestibular function showed neither a nystagmic pattern nor slow phase eye movements that were consistently modulated by the stimulus. No reliable measurements of slow phase velocity could be obtained.

Head motion

This condition was assessed in Case 1 only. No slow phase compensatory eye movements could be identified during oscillation. Mean eye position in the orbit was approximately sinusoidally modulated, adopting an anticompensatory position in the orbit (i.e., in the same direction as head motion), 90° phase advanced with respect to the position signal from the helmet. These changes in position were achieved by rapid successions of small saccades in the direction of head movement. Instructing the patient to imagine an earth-fixed target induced low velocity compensatory eye movements (gain = 0.09) and small saccades in rapid succession which were both in the direction opposite to head turn.

TABLE 1 GAIN OF THE COMPENSATORY EYE MOVEMENT DURING SINUSOIDAL MOTION (0.3 Hz) IN THE DARK

Subjects	*Head motion*	*Trunk motion*
Case 1	Nil (no instruction) 0.09 (imagining)	Nil
Case 2	Not done	Nil
(Absent vestibular function)	0.37, SD 0.16 (n = 8)	0.24, SD 0.09 (n = 9)
Normal controls	0.82, SD 0.13 (n = 9)	0.02, SD 0.03 (n = 13)

Table 1 summarizes the COR results in the dark in the 2 patients studied and compares them with a group of patients with absent vestibular function and normal controls previously studied by us with identical techniques (Bronstein and Hood, 1986, 1987). COR gain during trunk motion and head motion is considerably higher in the patients with pure vestibular loss than in the 2 patients with defective pursuit-vestibular function. The negligible trunk motion gain in the 2 patients with combined pursuit-vestibular failure was similar to that found amongst normal subjects. The high gain found during head motion in the normal control group is of course due to the presence of the VOR.

Experiments in the light

Fig. 3 shows that the eye movements evoked by target motion (SP-OKN), rotation en bloc and head on trunk rotation in the presence of optic fixation, were remarkaby similar. Slow phase eye movements were unable to match the relative velocity of the

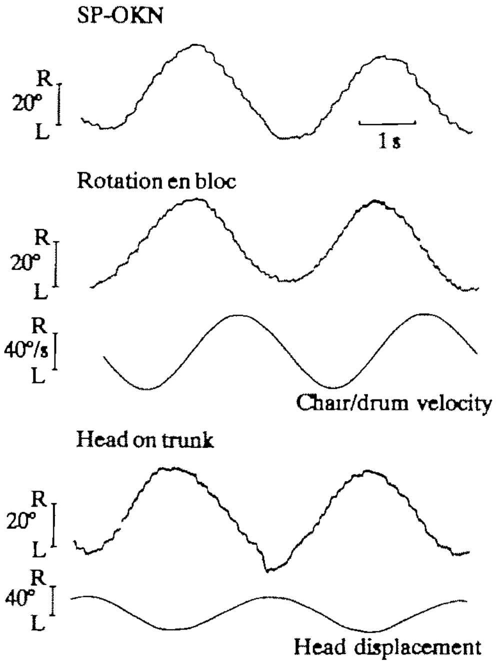

FIG 3. Compensatory eye movements during active visual fixation in response to sinusoidal oscillation of the OKN drum (SP-OKN), head and trunk (en bloc) and head alone (on trunk) in a patient with absent vestibular function and cerebellar dysfunction Note the similar 'cogwheel' appearance of the response under all testing conditions

fixation target so that they were frequently supplemented by small catch-up saccades. Additional recordings of smooth pursuit to a small laser target moving at the same frequency and velocity (not shown) had a similar appearance.

Table 2 summarizes the gain values for the 2 patients in the 3 test conditions. A two-way analysis of variance test (Friedmann) confirmed that neither in Case 1 ($P = 0.15$) nor in Case 2 ($P = 0.56$) was there a significant difference among the gain values obtained in the 3 test conditions. Thus it can reasonably be assumed that in these patients neither an input from the neck nor from the vestibular apparatus contributed to generate compensatory eye movements at this frequency; the only source of (defective) slow phase compensatory movements was the SP-OKN system.

TABLE 2 GAIN OF THE COMPENSATORY EYE MOVEMENT DURING SINUSOIDAL MOTION (0.3 Hz) IN THE LIGHT

Case	*Target motion (SP-OKN)*	*Rotation en bloc (head and trunk)*	*Head rotation (head on trunk)*
1	0.45, SD 0.05	0.41, SD 0.11	0.39, SD 0.06
2	0.43, SD 0.04	0.45, SD 0.03	0.46, SD 0.06

DISCUSSION

There is general agreement in the literature that in normal man and in higher mammals the COR, as assessed by trunk movements, is negligible and may have no clearly identified function (Dichgans *et al.*, 1973; Barnes and Forbat, 1979; Fuller, 1980; Baker *et al.*, 1982; Bronstein and Hood, 1986). In fact, near perfect retinal stability during head movements is achieved with the VOR alone whereas if the neck-eye loop were robust, unwanted eye movements might arise during movements of the trunk around the head. However, since the gain of the VOR in the dark elicited by oscillation of the head with respect to the trunk is 20–30% higher than that of the VOR obtained by oscillation en bloc at identical frequencies, it is accepted that the simultaneous activation of neck receptors 'switches in' preprogrammed oculomotor activity associated with the more natural movement of the head on the shoulders (Barnes and Forbat, 1979; Bronstein and Hood, 1986).

Following bilateral labyrinthectomy (monkey; Dichgans *et al.*, 1973) or plugging of the semicircular canals (cat; Baker *et al.*, 1982), the gain of the COR rises considerably and is able to generate about a third of the compensatory eye movements needed during natural head movements. Preprogrammed oculomotor activity is also associated with the COR since, in the absence of vestibular function, head movements generate compensatory eye movements of higher gain and of a wider frequency range than those elicited by trunk motion (Dichgans *et al.*, 1973; Kasai and Zee, 1978; Barnes, 1979; Takahashi *et al.*, 1981; Bronstein and Hood, 1986, 1987). Admittedly, in some clinical studies not all patients with absent vestibular function were found to have a significantly enhanced trunk motion COR (Barnes, 1979; Leopold *et al.*, 1983; Chambers *et al.*, 1985), but considerable differences in the experimental techniques used and in the instructions given to the subjects may well be responsible for this variability. However, there is overwhelming evidence that the more natural head motion COR generates compensatory eye movements of a gain similar to that reported in animal studies (Kasai and Zee, 1978; Takahashi *et al.*, 1981, 1989; Bles *et al.*, 1984; Bronstein and Hood, 1986; Leigh *et al.*, 1987).

The fact that the 2 patients described here with absent vestibular function and deranged SP-OKN eye movements did not have an enhanced COR cannot be explained by differences in the experimental design. In this respect, we compared the records of the 2 patients under study with 15 patients with pure absence of vestibular function (12 cases reported previously, plus 3 additional cases recently tested) and established that the COR, under identical experimental conditions, was strikingly different in these two groups of patients. Therefore, it would appear that the failure of the COR to enhance and to contribute to gaze stability during head movements in the 2 patients reported in this study may be of significance in understanding the structures mediating adaptive changes in the neck-eye loop.

The finding that the changes in the slow phase COR which normally occur after vestibular loss may not develop in the presence of a concurrent cerebellar lesion suggests that they may be subserved by similar mechanisms to those mediating other adaptive processes in the oculomotor system. In particular, it has been shown that the plastic adaptation induced in the VOR by optical devices is abolished by removal of the vestibulocerebellum (Robinson, 1976; Lisberger *et al.*, 1984). Alternatively, some pathways carrying slow phase COR signals might pass through the cerebellum and have been directly involved in our patients. For instance, it has been shown that some vestibular nuclei cells in which vestibular and neck signals interact receive their input from the neck via the cerebellum (Boyle and Pompeiano, 1981). A third possibility is that the potentiation of the neck-eye reflex is mediated through SP-OKN pathways or mechanisms. In the absence of the VOR, head movements in the light provide the retinal information required to initiate compensatory eye movements of SP-OKN origin. It is possible that, on repetition of head movements, such eye movements might begin to be triggered by neck stimulation alone, even in the absence of a concomitant retinal signal. A lesion affecting SP-OKN pathways at a cortical, cerebellar or brainstem level, however, would preclude the development of such a mechanism. Thus it is not clear whether the lack of COR enhancement in our patients is due to (1) damage of some structure specifically involved in oculomotor adaptive processes (e.g., the cerebellum), (2) direct interruption of slow phase COR pathways, or (3) derangement of SP-OKN eye movements. Unfortunately, neural damage in degenerative diseases of the type affecting our patients is widespread so that a correlation between abnormal function and the site of a lesion cannot be made with precision. A definitive answer to the problem of which mechanisms mediate adaptive changes in the COR may have to await studies with more restricted lesions.

In addition to generating compensatory slow phase eye movements, vestibular stimulation gives rise to saccadic eye movements (fast or quick components of nystagmus), which shift gaze in the direction of the ongoing head movement, the so-called 'anticompensatory' direction. These fast eye movements are thought to play a role in visual scanning of the environment and target acquisition during head movements (Jones, 1964; Barnes, 1975; Roucoux *et al.*, 1981). In patients with absent vestibular function, neck torsion produces vigorous saccades in the direction of the relative head movement, which suggests that the COR takes over the 'anticompensatory' function of the VOR (Frenzel, 1928; Bronstein and Hood, 1986). These fast components are under strong central regulation and can be suppressed by a verbal instruction to imagine an earth-fixed target during neck torsion, as illustrated in fig. 1, presumably by 'switching-in' a purely compensatory strategy (Bronstein and Hood, 1986). The patients reported here showed a preserved ability to generate quick, anticompensatory components of the COR and the patient with less widespread CNS involvement (Case 1, fig. 1) was also able to shift from a spontaneously anticompensatory COR mode to a compensatory one. However, due to the lack of slow phase compensatory eye movements this was achieved by a succession of small saccades interspersed with weak slow eye movements in the same direction, a pattern which was remarkably similar to the patient's SP-OKN movements. The findings would therefore suggest, first, that the cervical input had preserved access to fast eye movement mechanisms and that both patients may use the anticompensatory function of the COR to shift the eyes in the direction of an ongoing head movement; secondly, that the patient illustrated was probably using a cervical signal in order to aid fixation on a target of interest by means of quick components directed in the compensatory direction, a mechanism already demonstrated in patients with pure absence of vestibular function although to a lesser degree (Kasai and Zee, 1978), and thirdly, that the process mediating the switching from a compensatory to an anticompensatory strategy seems to be independent of the cerebellum. It would be likely that such a process, as suggested by Jürgens *et al.* (1982), requires the integrity of the cerebral cortex. The fact that Case 2, with more widespread CNS involvement, had lost the ability to shift strategies on verbal instruction would be in support of that view.

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(Received September 19, 1989. Revised December 19, 1989. Accepted January 2, 1990)

Brain (1991), 114, 13–49

NEUROACANTHOCYTOSIS

A CLINICAL, HAEMATOLOGICAL AND PATHOLOGICAL STUDY OF 19 CASES

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SUMMARY

Nineteen cases are described, including 12 cases from three different families and 7 nonfamilial cases, in which multisystem neurological disease was associated with acanthocytosis in peripheral blood and normal plasma lipoproteins. Mild acanthocytosis can easily be overlooked, and scanning electron microscopy may be helpful. Some neurologically asymptomatic relatives with significant acanthocytosis were identified during family screening, including some who were clinically affected.

The mean age of onset was 32 (range 8–62) yrs and the clinical course was usually progressive but there was marked phenotypic variation. Cognitive impairment, psychiatric features and organic personality change occurred in over half the cases, and more than one-third had seizures. Orofaciolingual involuntary movements and pseudobulbar disturbance commonly caused dysphagia and dysarthria that was sometimes severe, but biting of the lips or tongue was rarely seen. Chorea was seen in almost all symptomatic cases but dystonia, tics, involuntary vocalizations and akinetic-rigid features also occurred. Two cases had no movement disorder at all. Computerized tomography often demonstrated cerebral atrophy. Caudate atrophy was seen less commonly, and nonspecific focal and symmetric signal abnormalities from the caudate or lentiform nuclei were seen by magnetic resonance imaging in 3 out of 4 cases.

Depression or absence of tendon reflexes was noted in 13 cases and neurophysiological abnormalities often indicated an axonal neuropathy. Sural nerve biopsies from 3 cases showed evidence of a chronic axonal neuropathy with prominent regenerative activity, predominantly affecting the large diameter myelinated fibres. Serum creatine kinase activity was increased in 11 cases but without clinical evidence of a myopathy.

Postmortem neuropathological examination in 1 case revealed extensive neuronal loss and gliosis affecting the corpus striatum, pallidum, and the substantia nigra, especially the pars reticulata. The cerebral cortex appeared spared and the spinal cord showed no evidence of anterior horn cell loss.

Two examples of the McLeod phenotype, an X-linked abnormality of expression of Kell blood group antigens, were identified in a single family and included 1 female. The genetics of neuroacanthocytosis are unclear and probably heterogeneous, but the available pedigree data and the association with the McLeod phenotype suggest that there may be a locus for this disorder on the short arm of the X chromosome.

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INTRODUCTION

Acanthocytosis refers to the presence of abnormal erythrocytes bearing spiky projections (acantha, thorn, Greek) in peripheral blood. The first recognized neurological association was that described by Bassen and Kornzweig (1950), whose eponymous syndrome results from inherited abetalipoproteinaemia and was sometimes referred to as hereditary acanthocytosis. However, in the last 20 years cases with acanthocytosis and neurological impairment have been reported without lipoprotein abnormalities (*see* Hardie, 1989).

The syndrome of chorea associated with acanthocytosis was initially described in two separate North American kindreds (Critchley *et al.*, 1967; Levine *et al.*, 1968), and about 30 cases have subsequently been reported. Descriptive terms often applied to this association are 'familial amyotrophic chorea with acanthocytosis' (Kito *et al.*, 1980; Gross *et al.*, 1985) or 'chorea-acanthocytosis' (Ohnishi *et al.*, 1981; Sakai *et al.*, 1981; Limos *et al.*, 1982), emphasizing the commonest features of chorea, often involving the mouth and severe enough to result in mutilation, with neurogenic muscle wasting and areflexia. Because chorea does not occur universally, other designations have included 'familial neuroacanthocytosis' (Yamamoto *et al.*, 1982) or the 'Levine-Critchley syndrome' (Sakai *et al.*, 1985). Many patients have affected relatives, but no consistent pattern of inheritance has been identified.

A further inherited disorder associated with acanthocytosis is a rare X-linked abnormality of expression of the Kell blood group antigens known as the McLeod syndrome. Male subjects whose erythrocyte membranes express the McLeod phenotype always have elevated serum creatine kinase (CK) levels of 1000–2500 IU/l, and a benign X-linked myopathy has been described (Swash *et al.*, 1983). Reference has also been made to the coexistence of chorea and areflexia in certain male subjects with the McLeod syndrome (Schwartz *et al.*, 1982; Marsh, 1983), although the clinical features of these patients have not been described in detail.

Despite its apparent rarity, we have identified 7 nonfamilial cases of movement disorder with acanthocytosis in the UK in the last 6 years, together with a sibling pair and 10 affected members from two other families. Here we describe the wide spectrum of clinical features encountered in this series, together with the results of detailed haematological, biochemical, neurophysiological and neuropsychological evaluations. The results of neuropathological examination of 1 autopsy case and 2 sural nerve biopsies are also reported.

PATIENTS AND METHODS

Patients

The 19 cases consisted of 10 males and 9 females between the ages of 14 and 67 yrs All cases had acanthocytosis with neurological symptoms and/or abnormal neurological signs. There were no cases with parental consanguinity and all originated from the UK. Four cases belonged to family H and 6 to family L; their pedigrees are shown in fig. 1. Two brothers from family L were the subject of a short published report (Lowe *et al.*, 1977). The 2 siblings of family B were both affected, and 7 sporadic cases were identified. Blood samples were collected from as many asymptomatic family members as possible, who were also examined neurologically. Detailed case reports are included in the Appendix.

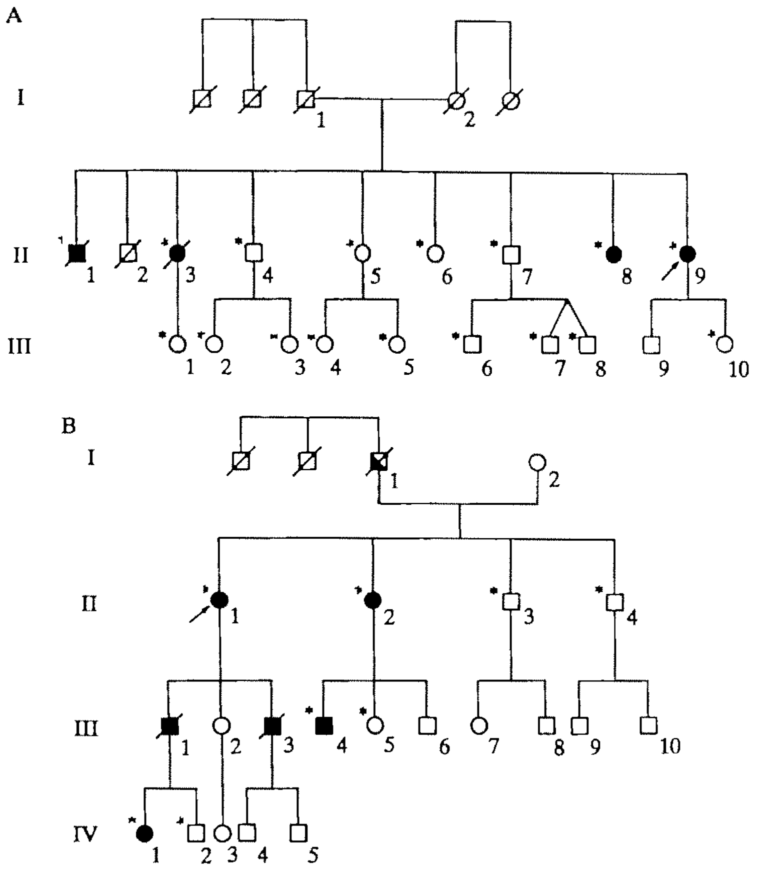

FIG 1 Pedigree of families H (A) and L (B). Arrow = index case, circle = female, square = male, filled symbols indicate neurological disease, * = examined by us, open symbols unaffected, half-filled = probably affected by history, open with * indicates neurologically normal on examination by us and no acanthocytes on blood film.

Methods

Haematology

Blood was collected into tubes containing ethylene-diamine-tetracetic acid (EDTA) and in addition, where possible, a blood film was made from fresh blood without EDTA contact. When this was not possible, blood films were made from EDTA-containing samples within 1 h of collection. Each sample was processed by a Coulter S-Plus JR or STKR and the blood count parameters obtained. The dried blood films were stained with May-Grunwald Giemsa stain at pH 6.8 and subsequently examined by light microscopy at ×1000 magnification under oil immersion. Care was taken to examine them at a point where the film was only one cell thick and with minimal space between the cells. In each case 500 cells were counted and the proportions of acanthocytes and echinocytes estimated.

For the purposes of this study an acanthocyte was defined as a dense, slightly contracted red cell which had a number of irregularly spaced thorny surface projections, often with terminal bulbs (Brecher and Bessis, 1972). Echinocytes also have an abnormal cell surface, but with more abundant and evenly distributed surface projections that have a much broader base in relation to their length. We regarded an acanthocyte count of >3% as significant.

A small amount of blood was also used for examination by scanning electron microscopy (SEM). Red cells were fixed in a 1% solution of glutaraldehyde in phosphate-buffered saline at neutral pH, and stored at 4° C until the time of examination. The red cells were absorbed onto tissue paper and gold-coated with an Emscope Sputter Coater. They were then examined in a Jeol 100 C electron microscope (with ASID unit) operating at 40 kV.

Blood group serology was carried out using standard manual techniques. Antiglobulin tests were read on a tile or in a tube using polyspecific antihuman globulin (Blood Products Laboratory (Diagnostics) Oxford, UK) or in capillary tubes using antihuman IgG reagents. ABO and Rh phenotypes were determined on all red cell samples, and selected anti-k and anti-K11 or anti-Kp^b sera were used to screen samples for the McLeod phenotype. Samples that showed the weak reactions characteristic of the McLeod phenotype were tested with all Kell and para-Kell antibodies.

Biochemistry

Blood samples were collected in standard heparinized tubes, and stored immediately at 4° C. Erythrocyte membranes were prepared from washed cells by osmotic lysis at 4° C with 20 mOsm Tris-HCl, pH 7.6 (Owen *et al.*, 1982), and their protein content was estimated with the Folin-Ciocalteau reagent (Lowry *et al.*, 1951) using bovine serum albumin as standard. Membrane lipids were extracted into isopropanol and chloroform (Rose and Oklander, 1965) and triplicate aliquots were taken for measurement of cholesterol, using a commercial kit (BCL, Lewes, East Sussex, UK), and of total phospholipids (Bartlett, 1959). The fatty acid composition of the phospholipids was determined by gas-liquid chromatography. A portion of the lipid extract was transmethylated by heating at 90° C for 1 h under N_2 in 14% (w/v) boron trifluoride in methanol (Kawata *et al.*, 1987). The fatty acid methyl esters were extracted into hexane and separated at 175° C on a 150 cm column of 10% EGSS-X on Gas Chrom P, 100/120 mesh; detection was by flame ionization. Individual phospholipid classes in the erythrocyte membranes were separated on silica gel H (Merck; BDH Chemicals Ltd, Poole, Dorset) using two-dimensional thin-layer chromatography as described previously (Owen *et al.*, 1982). The different fractions were detected with iodine vapour, scraped from the plate and measured as inorganic phosphorus after digestion with H_2SO_4 (Bartlett, 1959)

Peripheral nerve morphology

Sural nerve fascicular biopsy from a site immediately posterior to the lateral malleolus was obtained from 2 patients (Cases 4, 5) under local anaesthesia. Both sural nerves were taken within 72 h after death from the same site in Case 2. Segments of one sural and one femoral nerve were fixed in formalin for paraffin histology. The biopsied nerves were fixed for 3 h at 4° C in 2.5% glutaraldehyde in PIPES buffer (Baur and Stacey, 1977), and washed in buffer. The postmortem sural nerve was fixed overnight at 4° C in 3% glutaraldehyde in 0.05 M cacodylate buffer. After postfixation in 1% osmium tetroxide in the appropriate buffer, all nerves were dehydrated through graded concentrations of ethanol and embedded in Araldite using 1,2-epoxypropane as an intermediary.

Semithin sections (1 μm) for light microscopy were stained with thionin and acridine orange (Sievers, 1971), or methylene blue/basic fuchsin/azure II (Humphrey and Pittman, 1974). Thin sections for electron microscopy (EM) (Jeol 100 CX) were stained with 12.5% (or 25%, Case 2) methanolic uranyl acetate and lead citrate.

Some fascicles of the femoral nerve fixed in formalin were postfixed in OsO_4 and processed into Araldite. Single fibre preparations of this and each sural nerve were obtained by teasing in Araldite, employing the method described by Spencer and Thomas (1970); 100 fibres were isolated from the nerves of Cases 4 and 5. Several hundred fibres were examined from the sural nerve and femoral nerves of Case 2; about 50 fibres from each nerve were used for quantitation.

Myelinated fibre densities, fibre size/frequency distributions and internodal length/fibre diameter correlations were obtained from the biopsied nerves using a Kontron Videoplan image analysis system connected to a Leitz Ultraphot. Myelinated fibre counts in the sural nerve of Case 2 were obtained direct from the microscope; other quantitation was obtained using a Kontron MOP interfaced to an RML 380Z computer.

Postmortem examination

The brain and spinal cord were examined after several weeks' fixation in formalin. Blocks were taken from many regions of the cerebral hemispheres, the cerebellum, brainstem, many levels of spinal cord, cauda equina and sensory root ganglia. These were embedded in paraffin wax and, in addition to routine histological methods, were also stained with luxol fast blue-cresyl violet with silver by the Glees method and by the immunoperoxidase method for glial fibrillary acidic protein. Blocks were also taken from pectoralis major, deltoid and quadriceps muscles for histological examination.

RESULTS

The clinical features of each affected case are summarized in Table 1, and the results of relevant investigations in Tables 2 and 3. Further details are provided in the Appendix.

Clinical features

Behaviour disturbance, personality change and cognitive impairment occurred in over half the cases, and more than one-third had seizures. Depression, anxiety and obsessive-compulsive disorders each occurred in a few patients and 5 had received psychiatric care (Cases 1, 4, 11, 13, 16). Cases 4, 5, 15 and 16 all had a strikingly similar change in their behaviour; vague and distractable, they neglected their personal appearance and social skills and were no longer able to work. There was evidence of generalized intellectual deterioration in 11 cases, often with particular inefficiency in psychometric tests of attention and planning believed to be sensitive to frontal lobe dysfunction. The 2 youngest males (Cases 12, 17) had been thought to be dyslexic while at school.

Chorea affected the limbs, and sometimes the face, in almost all symptomatic patients, but in 4 (Cases 12, 17–19) the presenting and most striking movement disorder was dystonia rather than chorea, affecting speech as well as the face and limbs. Dystonia, especially of bulbar muscles, was also evident in 5 other cases. Repetitive stereotyped tics occurred in 8 cases. The 4 affected siblings of family H all gradually developed akinetic-rigid features, which eventually replaced chorea over the course of 20 years in Case 2, and parkinsonism also appeared in Case 11 and 2 sporadic cases. However, 2 of our younger cases (Cases 8, 10) had no movement disorder at all, perhaps because they were observed early in their clinical course.

Dysarthria was also common and usually represented a mixture of impairment by involutary movements (oromandibular and lingual dystonia and/or choreiform oro-faciolingual dyskinesia); the resulting pseudobulbar disturbance often caused dysphagia and was severe enough to cause virtual anarthria in 5 cases (Cases 1, 2, 14, 17, 19). Involuntary vocalizations occurred in 9 cases and comprised grunting, sucking, blowing, gasping, sighing or monosyllabic utterances but not recognizable words, often accompanied by belching, spitting, clicking or sniffing. Orofacial dyskinesia was present in 10 cases but persistent biting of the tongue, lips or buccal mucosa occurred in only 3 cases. A consistent complaint in 2 of these (Cases 15, 16) was difficulty keeping food in the mouth while chewing; this appeared to be due to involuntary dystonic protrusion of the tongue induced by mastication, forcing food out of the mouth and simultaneously making the tongue liable to be bitten.

Only 3 cases had significant muscle wasting or weakness (Cases 1, 2, 13). Areflexia was noted in 7 and hyporeflexia in 6 cases. Extensor plantar responses were present in 3 cases (Cases 1, 2, 5). No consistent sensory deficits were found. Cases 3 and 4 and their father had varying degrees of hearing impairment.

TABLE 1 CLINICAL FEATURES OF AFFECTED CASES

Case	*Sex*	*Age (yrs) of onset*	*Age (yrs) at last examination (or death*)*	*Dementia*	*Seizures*	*Psychiatric features*	*Orofacial dyskinesia*	*Dysphagia*	*Dysarthria*	*Movement disorder*	*Tendon reflexes*
Family H											
1	M	37	61*	+	+	+	O	+	+	P	O
2	F	39	61*	+	O	+	+	+	+V	CP	R
3	F	40	49	+	O	O	O	+	+V	CDPT	R
4	F	44	47	+	O	+	O	O	+V	CPT	O
Family L											
5	F	51	57	+	+	+	+	O	+	CT	O
6	F	–	56	O	O	O	O	O	O	C	+
7	M	24	31*	O	+	+	O	O	O	C	O
8	M	22	31*	O	+	O	O	O	O	O	O
9	M	–	27	O	O	O	O	O	O	CT	O
10	F	–	14	O	O	O	O	O	O	O	R
Family B											
11	F	12	24	+	O	+	+B	O	+	DPT	+
12	M	8	23	+	O	+	O	O	+	D	+
Sporadic cases											
13	M	44	51	+	+	+	+	+	+V	CP	O
14	M	62	67	+	O	+	+	O	+V	CDPT	+
15	F	22	25	+	+	+	+B	+	+V	CDT	+
16	F	28	36	+	+	+	+B	+	+V	CDT	R
17	M	8	18	+	O	O	+	+	+	D	+
18	M	33	36	O	O	O	+	+	+V	D	R
19	M	39	44	O	+	O	+	+	+V	D	R

B = tongue/lip biting; V = vocalizations, C = chorea, D = dystonia; P = parkinsonism; T = tics; O = absent, R = reduced; + = present

Haematology

None of the 19 cases or their examined relatives had a history of previous splenectomy, liver disease or haemolytic anaemia. Two patients (Cases 2, 3) had had previously documented iron deficiency anaemia. Otherwise all subjects had normal red cell indices and normal leucocyte and platelet counts. Polychromasia was not a prominent feature of the blood films and there was no evidence to suggest ongoing haemolysis.

Apart from Cases 1 and 2, all patients showed varying degrees of acanthocytosis by light microscopy on at least 2 occasions (Table 2). Previous laboratory reports of blood film microscopy from Case 2 (*see* Appendix) were strongly suggestive of the presence of acanthocytosis but this had not been appreciated at the time. On SEM the erythrocyte appearances were similar to those seen by light microscopy (fig. 2). There were no instances of discrepancy between the two techniques. However, in a few cases (Cases 2, 11, 14, 16) specific requests to the routine haematology laboratory to search for acanthocytes had yielded negative reports prior to diagnosis. The addition of plasma from several affected cases did not induce any morphological changes in fresh erythrocytes from normal subjects with compatible blood groups.

Only one neurologically normal relative, the daughter of Case 13, was found to have significant acanthocytosis during screening of 21 members of families H and L, both

TABLE 2. RESULTS OF INVESTIGATIONS

Case	*Per cent echinocytes**	*Per cent acanthocytes**	*CK (IU/l)*	*SAPs*	*EMG denervation*	*Cerebral atrophy (CT)*	*Caudate atrophy (CT)*
Family H							
1	NS	NS	–	R	+	+	+
2	NS	NS	–	–	–	+	+
3	10	10	401	N	A	+	+
4	10	25	–	R	+	A	A
Family L							
5	30	25	840	A	+	+	+
6	10	20	265	–	–	–	–
7	–	+	600	A	A	A	A
8	–	+	1980	–	–	A	A
9	20	10	4035	–	–	–	–
10	5	5	34	N	–	–	–
Family B							
11	30	15	109	R	A	A	A
12	10	12	103	–	–	–	–
Sporadic cases							
13	10	50	261	N	+	+	+
14	–	10	47	N	A	+	+
15	25	10	860	N	A	+	A
16	10	10	213	N	A	A	A
17	5	10	103	N	A	A	A
18	2	5	692	R	A	A	A
19	10	25	1980	R	+	+	A

* % echinocytes and acanthocytes by light microscopy. Normal serum creatinine kinase (CK) < 195 IU/l M, < 170 IU/l F; EMG = electromyography; R = reduced in amplitude; A = absent; + = present; – = not done; NS = not seen (but inadequate examination).

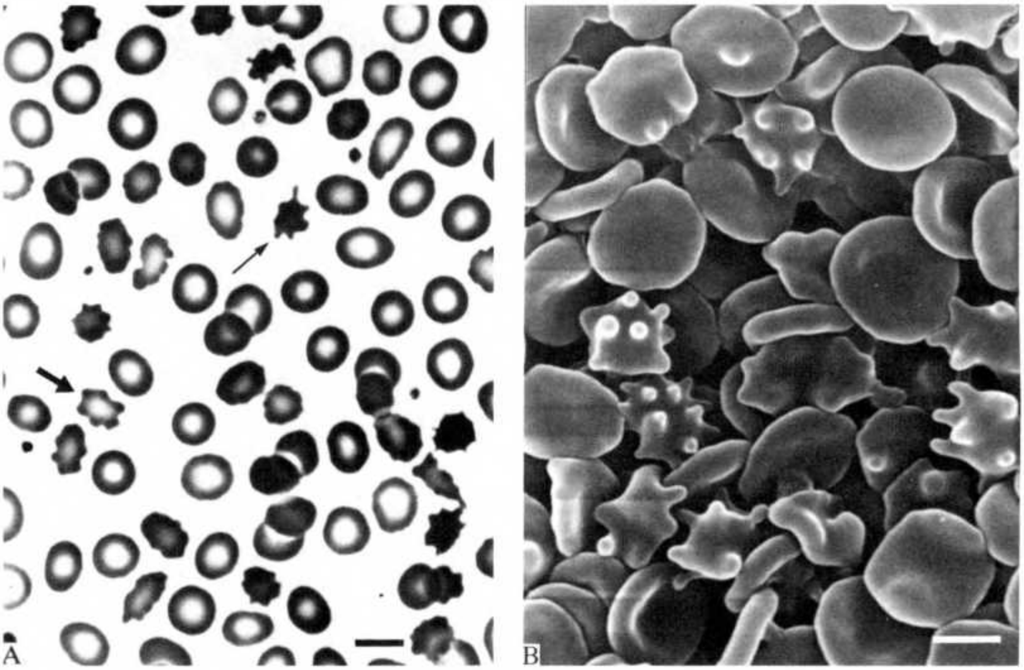

FIG. 2. Peripheral blood acanthocytosis. A, light microscopy. Bar = 7 μm. Both echinocytes (short arrow) and acanthocytes (long arrow) are seen. B, scanning electron micrograph. Bar = 4 μm.

parents of Cases 11 and 12, and 10 first degree relatives of sporadic cases. However, 4 of our cases (Cases 6, 9, 10, 12) were themselves asymptomatic but abnormal on examination, and were thus actually diagnosed while screening relatives of the index Cases 5, 7 and 11.

Blood group serology

Two examples of the McLeod phenotype were identified in living members of family L. The erythrocytes of Cases 5 (II.1) and 9 (III.4) both had weakness or absence of expression of the high-frequency Kell antigens (k, Kp^b, Js^b, K11, Ku) and para-Kell antigens (K12, K13, K14, K18, K22), compared with normal erythrocytes (Table 3). Their red cells were also negative for KL and one of its components, Kx. Karyotype analysis in Case 5 showed a normal XX female genotype.

In Case 6 (II.2), the sister of Case 5 and mother of Case 9, there was weakness of the para-Kell antigens and Kx antigen but Kell antigen expression was greater than that expected in the McLeod phenotype. Cases 7 and 8 were not available for testing, and the erythrocytes of Case 10 (IV.1) had slight depression of some Kell antigens of uncertain significance. Extended screening of asymptomatic members of family L identified no other abnormalities of the Kell phenotypes, and no other examples of the McLeod phenotype were identified amongst the other cases studied.

TABLE 3. BLOOD GROUP SEROLOGY AND SERUM CREATINE KINASE IN LIVING MEMBERS OF FAMILY L

		Kell antigens					*Para-Kell antigens*					*Markers*		*CK*
Pedigree no.	*Case*	*Kk*	*Kp*^{b}	*Js*^{b}	*K11*	*Ku*	*K12*	*K13*	*K14*	*K18*	*K22*	*Kx*	*KL*	*(IU/l)*
Affected family members with acanthocytosis														
II.1	5	− −	w	w	−	−	−	−	−	w	w	−	−	840
II.2	6	− +	+	+	+	+	w	+	w	w	w	w	−	265
III.4	9	− −	w	w	−	−	−	−	−	w	w	−	−	4035
IV.1	10	− +	w	+	+	w	+	+	+	/	+	+	w	141
Healthy family members with no acanthocytosis														
I.2		− +	+	+	+	+	+	+	+	/	+	+	+	182
II.3		− +	+	+	+	+	+	+	+	+	+	+	+	157
II.4		− +	+	+	+	+	+	+	+	+	+	+	+	121
III.2		− +	+	+	+	+	+	+	+	/	+	+	+	70
III.5		− +	+	+	+	+	+	+	+	/	+	+	+	89
III.6		− +	+	+	+	+	+	+	+	/	+	+	+	311
III.7		− +	+	+	+	+	+	+	+	/	+	+	/	80
III.8		− +	+	+	+	+	+	+	+	/	+	+	/	303
III.9		− +	+	+	+	+	+	+	+	/	+	+	/	131
IV.2		− +	+	+	+	+	+	+	+	/	+	+	+	220

All subjects were negative for Kp^a, Kp^c, Js^a, and K17; normal serum creatine kinase (CK) <195 IU/l M, <170 IU/l F; + = positive; − = negative; w = weakly positive; / = not done.

Biochemistry

Serum CK activity was within the normal range in at least 5 cases (Table 2). The highest value of 4035 IU/l was found in Case 9, the male with McLeod phenotype, and in only 2 other males (Cases 8, 19) was the CK level >1000 IU/l. Amongst healthy members of family L without acanthocytosis (Table 3), 2 males (III.6, III.8) had CK levels above the normal range and higher than one of the affected females (Case 6, II.2).

Serum lipoprotein electrophoresis was normal in all subjects, thus excluding any cases of hypo or abetalipoproteinaemia. The cholesterol and phospholipid contents per mg protein in erythrocyte membranes from healthy members of family H fell within the range for normal subjects previously determined (Owen *et al.*, 1982). The results for Cases 3 and 4 (II.8 and II.9 of family H) were not only 10−15% lower than the mean for the healthy family members but fell outside their range of values (Table 4). The corresponding values of the 2 daughters (III.1 and III.10) were also low but within the normal family range. However, the composition of the lipid constituents within the

TABLE 4. LIPID COMPOSITION OF ERYTHROCYTE MEMBRANES FROM LIVING MEMBERS OF FAMILY H

Case	*Cholesterol (nmol/mg protein)*	*Phospholipid (nmol/mg protein)*	*Cholesterol/ phospholipid (mol/mol)*	*Lecithin/ sphingomyelin (mol/mol)*
3 (II.8, F)	452	584	0.774	1.20
4 (II.9, F)	476	617	0.771	1.09
III.1, F	511	651	0.785	1.26
III.10, F	514	676	0.760	1.06
Healthy family members (Mean ± SD) (n = 11)	536 ±26	689 ±35	0.779 ±0.028	1.23 ±0.17

P25701

patients' membranes appeared normal when expressed as ratios or proportions rather than absolute amounts per mg membrane protein. The cholesterol/phospholipid and lecithin/sphingomyelin molar ratios were all within the normal range (Table 4), as were the proportions of the individual phospholipid classes and fatty acyl chains (data not shown). Similar results were also obtained for the lipid composition of erythrocyte membranes from Case 17, with cholesterol/phospholipid and lecithin/sphingomyelin molar ratios of 0.815 and 0.91 mol/mol, respectively.

Other investigations

Computerized tomography (CT) brain scans were normal in 7 cases, showed both generalized cerebral and focal caudate atrophy in 6, and cerebral atrophy alone in a further 2 cases (Table 2). Magnetic resonance imaging (MRI) of the brain in 3 out of 4 cases demonstrated focal symmetrical abnormalities in various parts of the basal ganglia, with increased signal on T_2 weighted sequences from the caudate (Case 15) or lentiform (Cases 14, 17) nuclei (fig. 3).

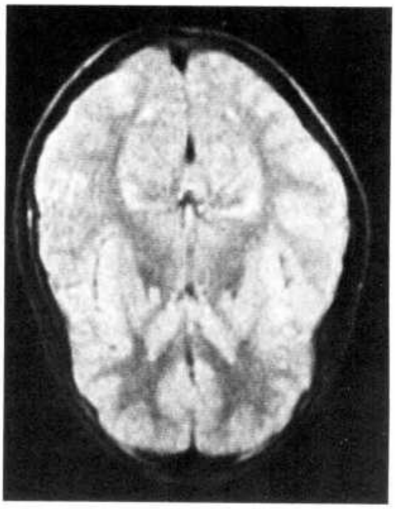

FIG. 3. Magnetic resonance T_2 weighted spin echo image (SE 2000/80 ms) of the brain in *Case 17* showing symmetric abnormally high signal from the medial pallidal segments.

Motor nerve conduction velocities (MNCVs) were normal in all 14 cases studied, but sensory action potentials (SAPs) were reduced or absent in 7 cases. Electromyography (EMG) showed evidence of denervation in 5 cases. Electroencephalography (EEG) was usually normal or showed nonspecific abnormalities; only 1 (Case 15) had focal EEG abnormalities.

Peripheral nerve morphology

Cases 4 and 5. Transverse sections of the nerve biopsy specimens showed a depletion of myelinated nerve fibres (fig. 4). Myelinated fibre density was reduced to 6836/mm^2 in Case 4, and to 3210/mm^2 in Case 5 (normal range 7500 – 10 000/mm^2, Jacobs and Love, 1985). Fibre diameter frequency histograms confirmed a selective reduction in

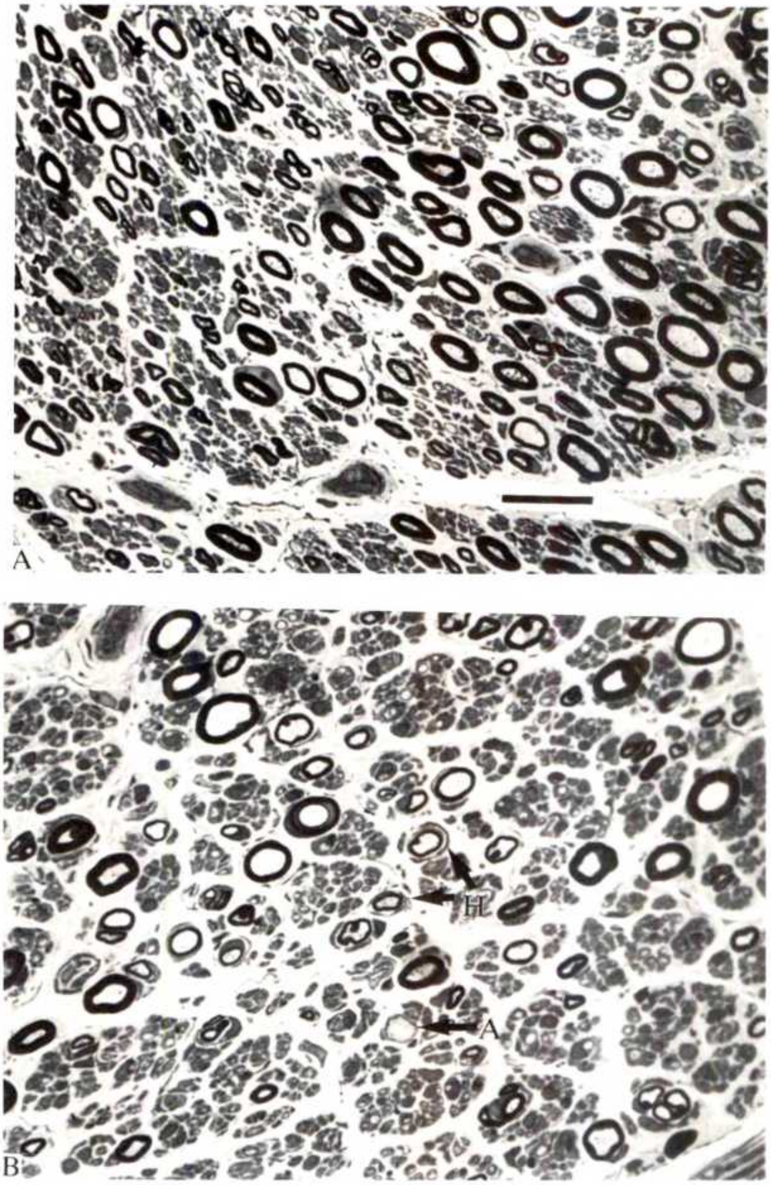

FIG. 4. Transverse sections of portions of sural nerve biopsy specimens. A, *Case 4*. Thionin and acridine orange. Bar = 20 μm. B, *Case 5*. Note hypertrophic changes of Schwann cells (H), and a giant axon (A) detailed in fig. 9. Thionin and acridine orange. Same scale as fig. 4A.

the large diameter myelinated fibre population in both patients (fig. 5). While no active fibre degeneration or remyelination was observed in the semithin sections, regenerative clusters were seen and many of the surviving myelinated fibres had abnormally thin myelin sheaths. Analysis of the internodal lengths of fibres in relation to fibre diameter

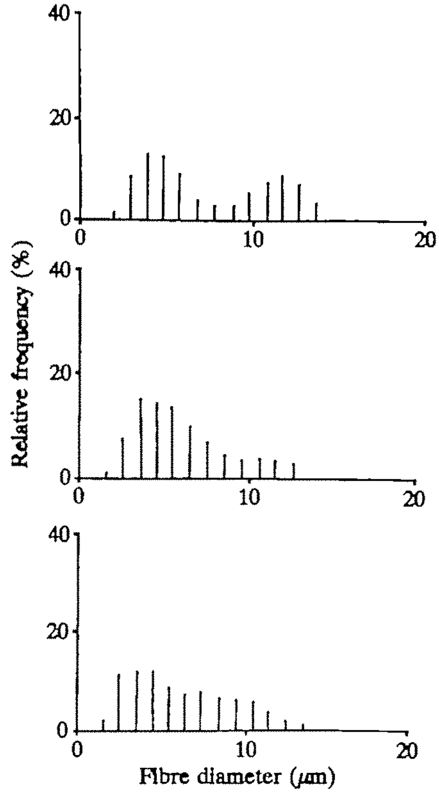

FIG. 5. Diameter-frequency distributions of myelinated nerve fibres in the sural nerve biopsies. The *upper* histogram shows the fibre distribution from a control subject while the *lower* two histograms are from *Cases 4 and 5*.

showed uniformly short internodal lengths in both patients (fig. 6), suggesting regeneration after axonal degeneration.

On EM both cases showed extensive chronic axonal degeneration with bands of Büngner and collagen pockets and also flat sheets of Schwann cell processes indicating unmyelinated fibre loss. Some fibres were seen in the early stages of demyelination or degeneration (fig. 7). There were also thinly myelinated fibres, some surrounded by excess Schwann cell processes forming hypertrophic whorls. In one case, occasional giant axons were seen in which the axon was distended by accumulations of disorganized neurofilaments (fig. 8). Some Schwann cells contained unusual inclusions possibly derived from mitochondria. The endoneurial blood vessels were surrounded by greatly thickened basal laminae. No inflammatory cells were found and the perineurium was normal.

Case 2. Paraffin histology of the femoral nerve from Case 2 showed no abnormality apart from some increase in the numbers of Schwann cell nuclei. In the sural nerve, there were several fascicles with increased numbers of Schwann cell nuclei. Three other fascicles were much more cellular, they had no clearly defined perineurium and appeared to contain thinly myelinated fibres. No inflammatory cells were seen in either nerve.

Transverse semithin sections of the sural nerve from the opposite side showed that most of the fibres had thin myelin sheaths relative to axon size, and many were in regenerative clusters (fig. 9). The density of myelinated fibres was moderately reduced

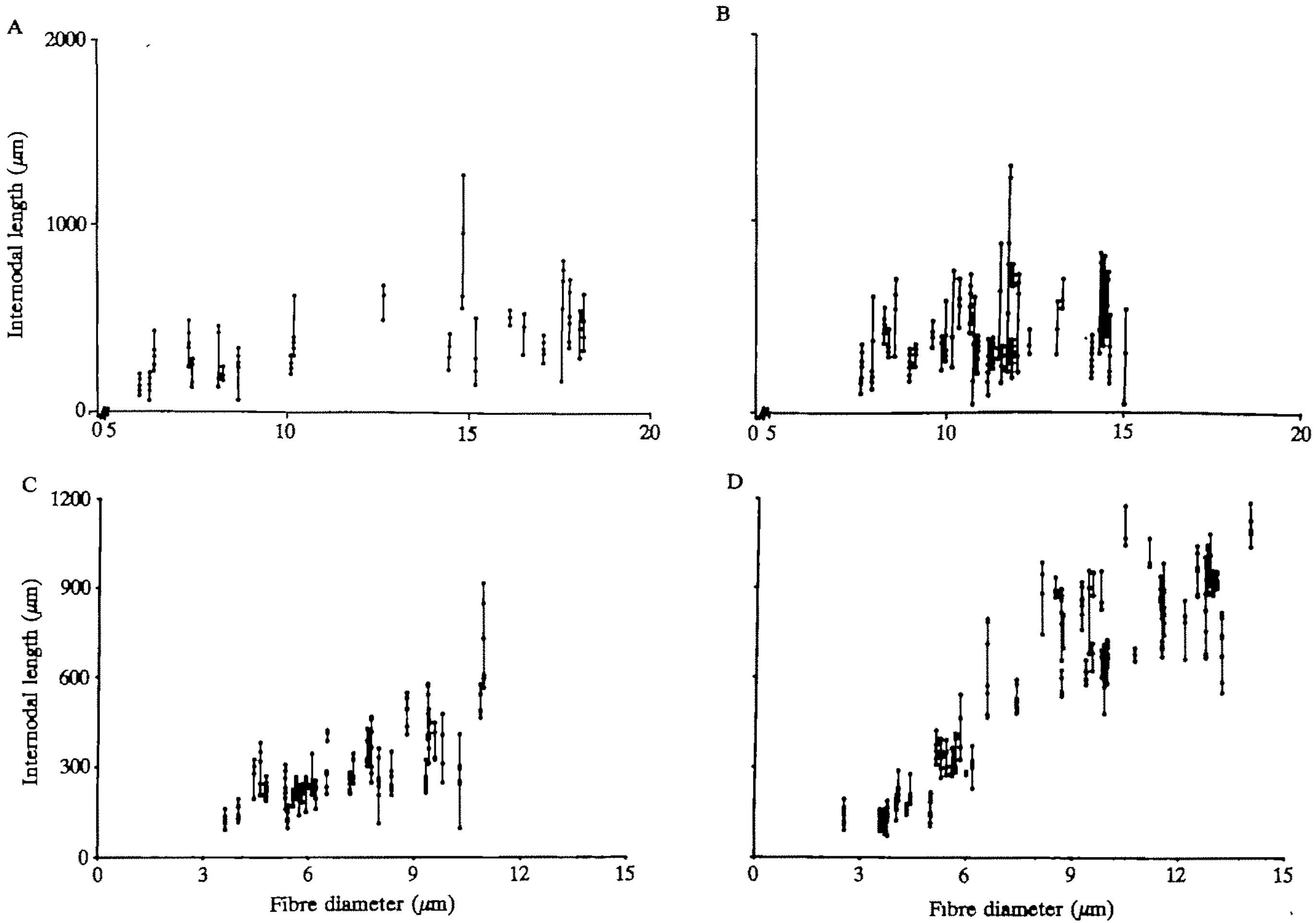

Fig. 6 Graphic representations of internodal lengths and fibre diameters of teased fibres from sural nerves. Lines join up values of internodal length from the same fibre. A, *Case 4*. B, *Case 5*. C, *Case 2*. In all fibres except one, the internodal lengths are relatively uniformly short and do not exceed 600 μm. D shows the linear relationship between internodal length and fibre diameters in a control nerve.

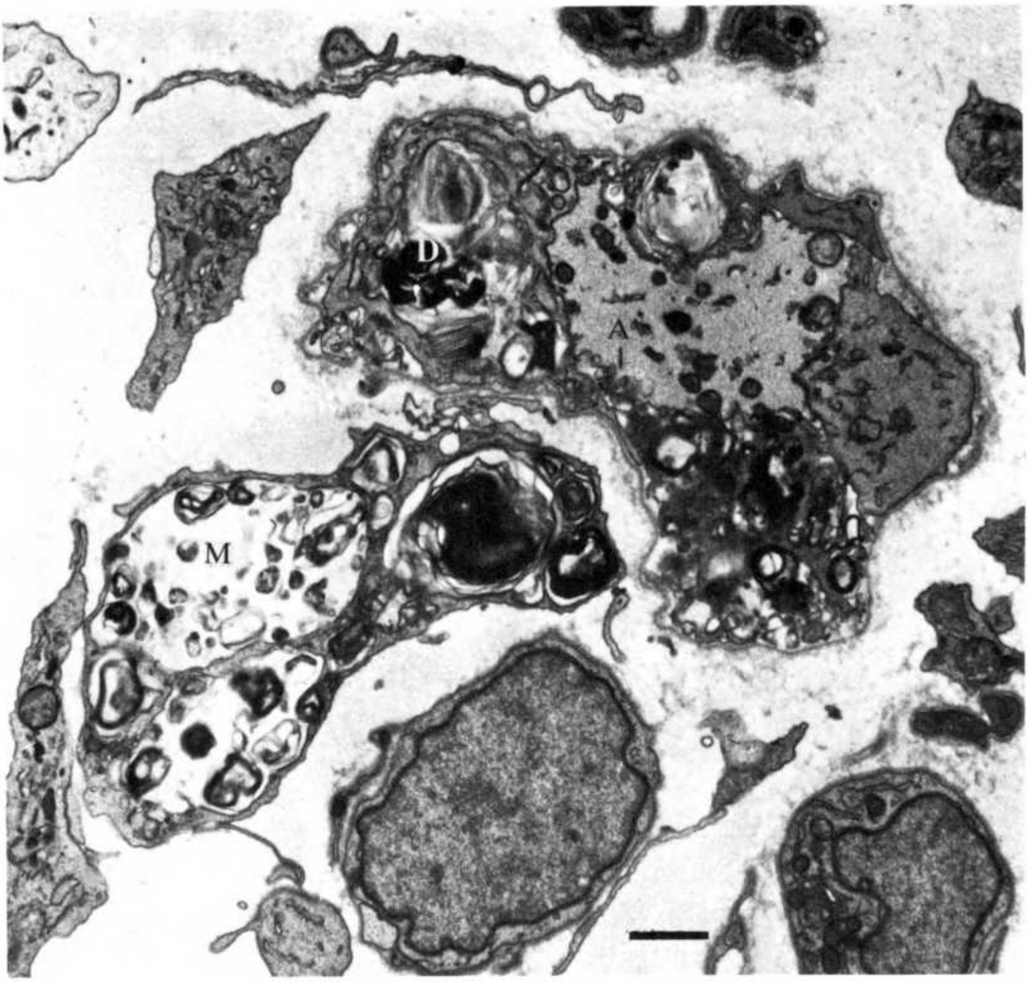

FIG. 7. Electron micrograph of sural nerve biopsy of *Case 5* showing macrophage (M), debris in Schwann cell (D) and demyelinated axon (A). Bar = 1 μm.

(5087/mm^2), but this figure includes many fibres in regenerative clusters. There were one or two very large and deeply stained axons. Endoneurial collagen was increased.

Teased fibre preparations of the sural nerve showed that almost all the fibres were regenerated, with uniformly short internodes (fig. 6c). In the femoral nerve there were some fibres with normal internodal lengths for fibre diameter, and others with short internodes. Examination of a large number of fibres from both nerves showed only one with a focal axonal swelling. The sural nerve was quite well preserved considering the delay in fixation after death. EM confirmed the presence of regenerated fibres both as groups of myelinated fibres or as single well-myelinated fibres surrounded by unmyelinated fibres. There was no ongoing axonal degeneration.

As in the other 2 cases there was evidence of loss of unmyelinated axons; some very

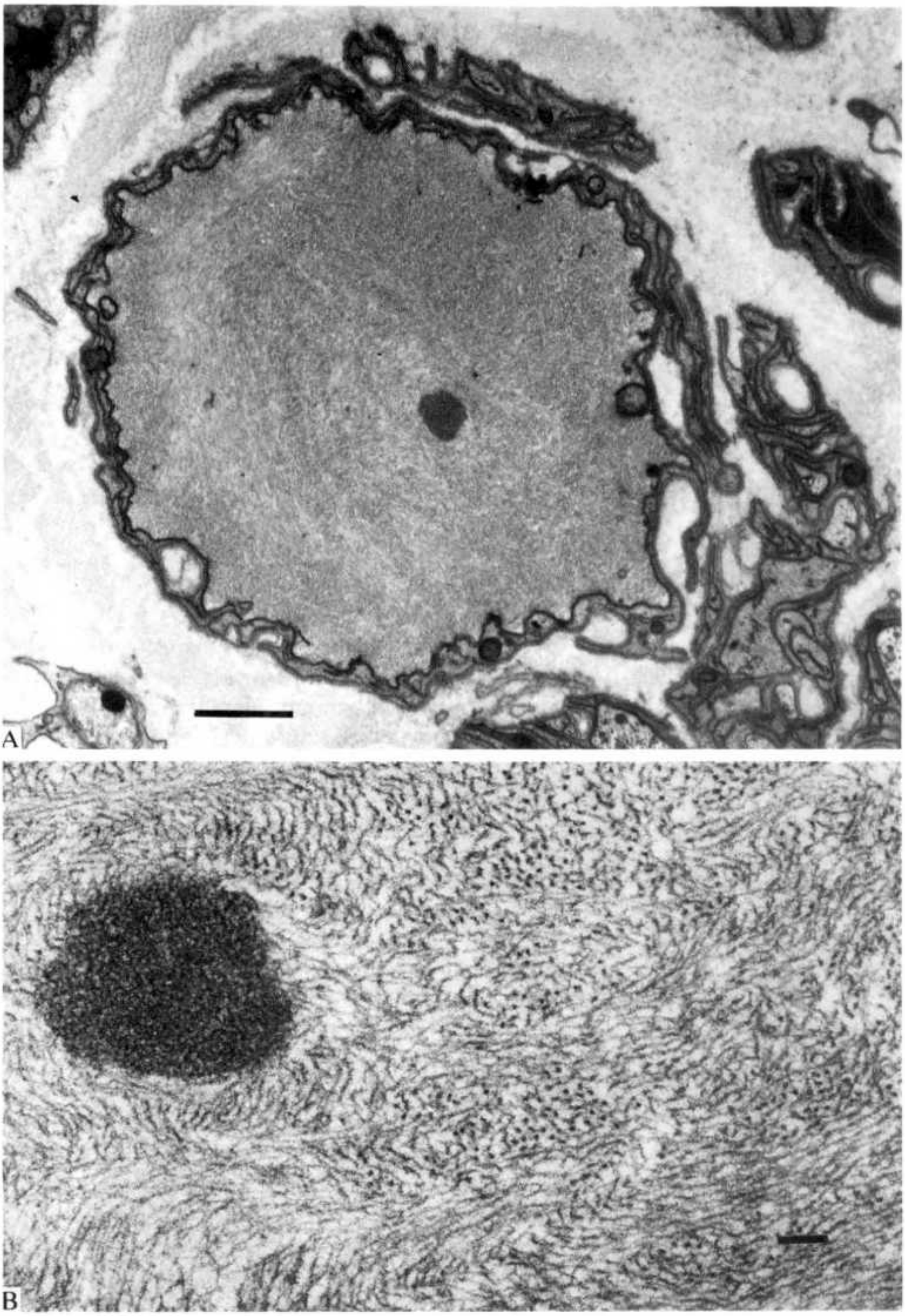

FIG. 8. A, electron micrograph of sural nerve biopsy of *Case 5* showing a giant axon. Bar = 1 μm. B, at a higher power the axon is seen to be distended by accumulations of neurofilaments. Bar = 0.1 μm.

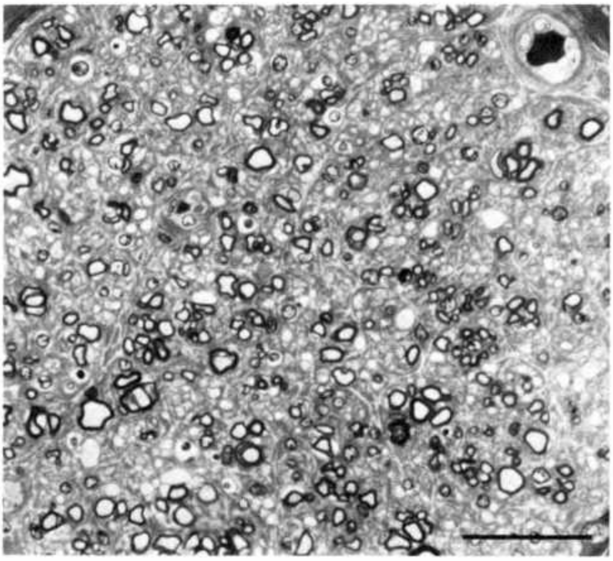

FIG. 9. *Case 2*. Araldite section (1 μm) of postmortem sural nerve showing many regenerative clusters. Bar = 100 μm.

small, probably regenerating, axons were found. A few enlarged axons were seen: one mildly enlarged myelinated fibre contained whorls of neurofilaments; several other large, probably unmyelinated, axons contained masses of smooth membranes in 1 case and another was filled with amorphous granular material with a peripheral rim of neurofilaments and mitochondria in various stages of degeneration.

Postmortem findings in Case 2

The main pathological changes outside the CNS included evidence of a recent operation for a fractured right femur, severe atheroma causing stenosis of the three main coronary arteries and a recent myocardial infarct at the apex of the left ventricle, and terminal bronchopneumonia. The muscles of both legs were severely wasted and there was marked inversion of both feet.

The formalin-fixed brain weighed 1235 g and the gyral pattern and meninges were normal. There was severe patchy occlusive atheroma of the arteries at the base, the left vertebral, basilar and left carotid arteries being most affected. The right vertebral artery was very small but patent and there was atheroma and stenosis of the right middle cerebral artery and its branches. Coronal slices showed moderate symmetric dilatation of the ventricles particularly affecting the frontal horns. There was very marked atrophy of the caudate, putamen and pallidum (figs 10, 11). These structures were symmetrically shrunken, rather soft and spongy in consistency and showed a brownish discoloration. In the putamen and thalamus there were many small lacunae. A number of infarcts of varying age and size were observed, ranging from one affecting the left posterior frontal cortex and underlying white matter of several weeks' duration to a large left temporo-occipital infarct particularly involving white matter and showing cavitation, haemosiderin pigmentation and surrounding scarring. Several old infarcts a few millimetres in diameter

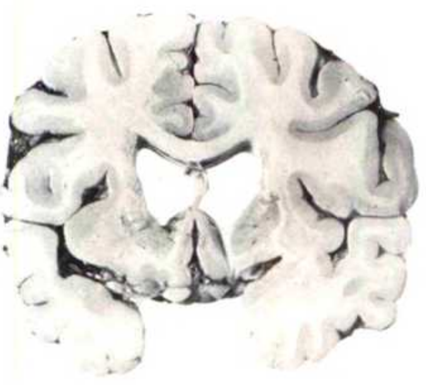

FIG. 10. *Case 2*. Coronal slice through the frontal and temporal lobes of the cerebral hemispheres. There is severe atrophy of the caudate nuclei and dilatation of the anterior horns of the lateral ventricles. Note the concave configuration and angulation of the lateral walls of the ventricles.

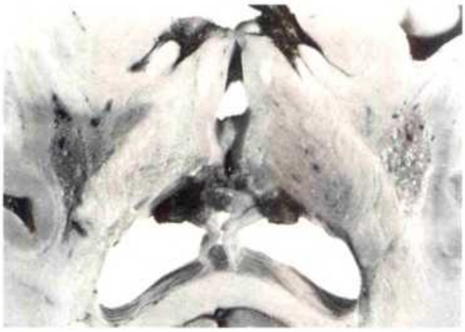

FIG. 11. *Case 2*. Close-up view of cerebral hemispheres at the level of the foramen of Monro and basal ganglia. There is very severe symmetric atrophy and sponginess of the putamen and pallidum which appeared darker than normal due to a brownish discoloration.

were present in the left thalamus and internal capsule and scattered in the centrum semiovale.

The aqueduct was widely patent and the substantia nigra markedly pale on both sides. The locus coeruleus was normally pigmented. The pons, medulla and cerebellum showed no abnormalities and the spinal cord was of normal appearance macroscopically. Both anterior and posterior nerve roots were of normal thickness and apparently well myelinated.

Histological examination. In the basal ganglia the caudate was virtually depleted of both large and small neurons. Very severe astrocytic gliosis was present, the tissue having a loose spongy appearance. The putamen and pallidum were slightly less severely depleted of neurons, and also showed very striking astrocytic gliosis (fig. 12). Small infarcts were present in the left putamen, internal capsule and thalamus as noted on macroscopic

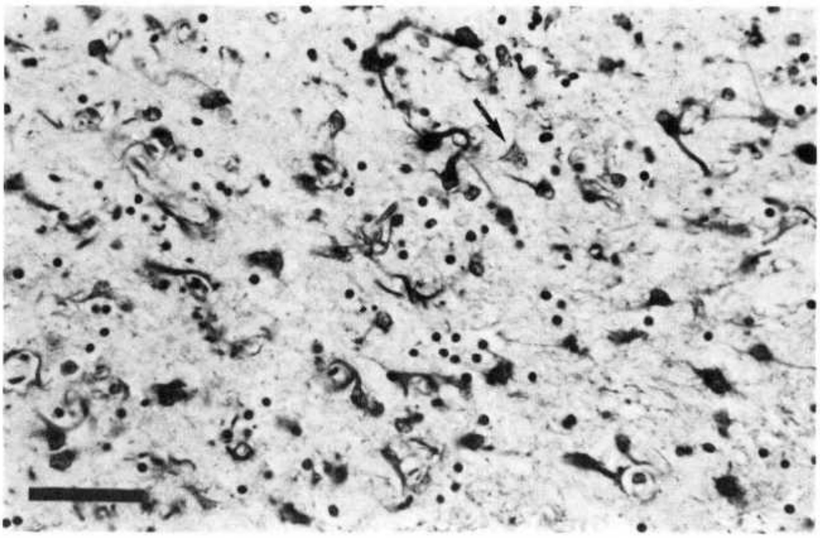

FIG. 12. *Case 2*. Section of putamen stained by the immunoperoxidase method for glial fibrillary acidic protein. Only an occasional neuron is preserved (arrow) while most other cells present are heavily stained astrocytes. Bar = 50 μm.

examination. Apart from the large old temporo-occipital infarct, marked perivascular rarefaction was present in the remainder of the hemispheres, with occasional pigment-laden macrophages lying in the spaces around the vessel walls. These changes were most marked in the centrum semiovale and in the basal ganglia. Vessels were hyalinized but not calcified. There was no amyloid. Atheroma was present in the cerebral arteries of larger calibre. The cortex including hippocampus and temporal lobe contained no evidence of neuritic plaques, neurofibrillary tangles or other neuronal inclusions.

In the cerebellum there was no evidence of Purkinje or granule cell loss and the dentate nuclei were well populated; neurons contained abundant lipofuscin. Small arteries in the white matter and especially in the dentate showed some thickening and hyalinization of their walls. There was marked loss of nerve cells in the substantia nigra and abundant extraneuronal pigment together with severe gliosis (fig. 13). The cell loss was virtually complete in the pars reticulata and less marked with relative preservation of pigmented neurons in the medial zona compacta. The population of pigmented neurons in the locus coeruleus was within normal limits and there was no extraneuronal pigment. A careful search was made for Lewy bodies in brainstem and cortex but none was identified. The olivary nucleus and ascending and descending fibres in the medulla were normal in appearance.

Anterior horns of the spinal cord appeared to contain a normal number of nerve cells, although this was not studied quantitatively. The grey matter was not gliotic. Silver preparations of anterior and posterior nerve roots showed no axonal loss and a normal population of myelinated fibres was present at all levels studied. Dorsal root ganglia from cervical and lumbar levels contained occasional Nageotte nodules in keeping with the patient's age. Changes indicative of partial motor denervation were present in all

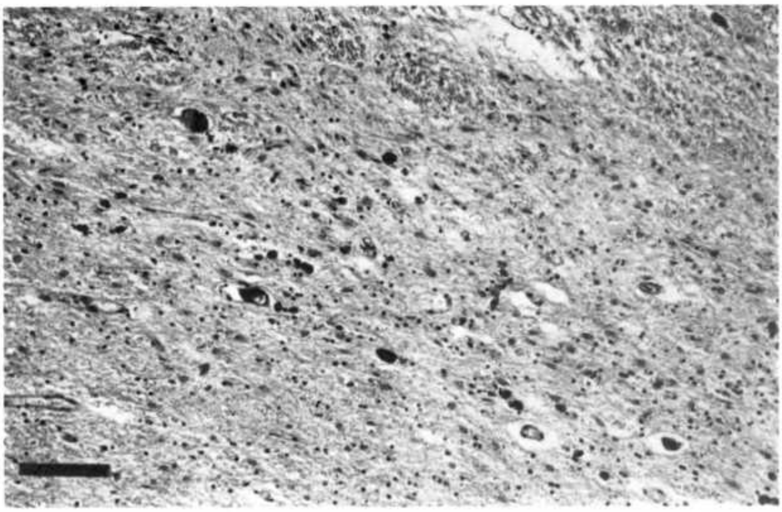

FIG. 13. *Case 2*. Section of pars compacta of substantia nigra. Only two or three intact neurons are seen in this field in which there is also abundant extraneuronal pigment as well as marked astrocytic gliosis. Haematoxylin and eosin. Bar = 100 μm.

muscles examined but were most severe in quadriceps where, in addition to the presence of many small angulated fibres, there was also fascicular atrophy.

DISCUSSION

Clinical features

In 1967 a 'new hereditary acanthocytosis syndrome' was described (Estes *et al.*, 1967) in a New England family of 15 members. Nine had significant (defined as $>1\%$) acanthocytosis in peripheral blood and a spectrum of neurological abnormalities, including 'muscular atrophy of the Charcot-Marie-Tooth type, neck and shoulder girdle atrophy, choreiform movements of the Huntington's type, dementia and nonfocal grand mal seizures'. Although these patients were stated to have normal serum lipid concentrations, it is confusing that the brother of the proband had previously been reported to have steatorrhoea, hypobetalipoproteinaemia and acanthocytosis (Kuo and Bassett, 1962) and he later developed a paranoid psychosis with generalized muscle wasting and weakness and involuntary movements of the limbs (Rovito and Pirone, 1963).

The clinical features of the 50-yr-old proband were detailed by Levine *et al.* (1968). The development at 27 yrs of progressive weakness, wasting and involuntary movements of the limbs was followed by seizures and dementia. Chorea also involved the face and tongue. Tendon reflexes were absent and sensation was impaired distally. Muscle biopsy was compatible with denervation. The brother referred to above was described as similarly affected, and 17 other family members were listed as having various neurological abnormalities. However, numerous clinical and haematological discrepancies between the reports of Estes *et al.* (1967) and Levine *et al.* (1968) must cast considerable doubt about the true extent of involvement of family members other than the 2 brothers, and whether the proband's brother had a lipid abnormality remains uncertain.

A syndrome of acanthocytosis, involuntary vocalizations and mutilating orofacial dyskinesia was reported by Critchley *et al.* (1967, 1968) in a 29-yr-old man with normal vitamin E and lipid studies. He frequently bit his lips and tongue, was dysarthric and made grunting and sucking noises. A wide spectrum of involuntary movements also affected the limbs including tics, dystonic and choreiform movements, and he had originally been given a tentative diagnosis of Tourette syndrome. Hypotonia and areflexia were also present although there was no muscle wasting and muscle biopsy was normal.

Three siblings had already died at the age of 26–31 yrs having had similar involuntary movements, a fourth was areflexic with mild chorea and acanthocytosis, and 2 of them had had seizures. Two of the 4 healthy siblings had 10% acanthocytosis alone (Critchley, 1974), 1 of whose children appeared to have Friedreich's ataxia but no significant acanthocytosis. Abnormalities of fat absorption and serum vitamin E and lipid concentrations were excluded in this case, a niece of the proband. These authors were careful to exclude artefactual red cell abnormalities and only 1 of over 30 other healthy family members screened using phase contrast microscopy had acanthocytosis. Critchley *et al.* (1970) subsequently described very similar findings in a young woman without muscle wasting or weakness and with normal findings on EMG and nerve conduction studies. She later committed suicide and a detailed autopsy was not carried out (Critchley, 1971).

Aminoff (1972) reviewed the association of acanthocytosis and neurological disorder while reporting 2 brothers with orofacial and limb chorea, areflexia, normal lipids and clinical, electrophysiological and histological evidence of neurogenic muscle disease. Since then at least a further 30 cases have been described, in various degrees of detail, in the English literature. According to Oshima *et al.* (1985), more than 50 cases in over 24 families were described in the Japanese literature between 1979 and 1983, but details of the majority are not available. Most of those cases were restricted to the southern part of Japan and parental consanguinity was common.

The salient clinical features of all previously fully reported cases are summarized in Table 5; these are similar to the range reported here. Lipid studies were normal in all except 1 kindred with borderline hypobetalipoproteinaemia (Kito *et al.*, 1980) which could have been coincidental. Amongst the fully reported cases, 18 were male and 8 female; in the present series, 10 were male and 9 female. The mean age of onset in the reported cases was 32.7 (range 13–58) yrs; amongst our patients it was 32.1 (range 8–62) yrs. Eight of the total 45 patients had died, after a mean duration of illness of 13.9 (range 7–24) yrs; the remainder were alive after a mean of 8.7 (range 3–33) yrs.

Dementia was clinically evident in 7 of 24 reported cases, and in 12 of our 19 patients. Our experience, and that of others, suggests that milder cognitive impairment identified by detailed neuropsychological assessment may be more frequent (Medalia *et al.*, 1989). Furthermore, psychiatric symptoms are common although varied. Depression, anxiety paranoid delusions and obsessive-compulsive features may all occur, but the most consistent symptoms are those of organic personality change suggestive of frontal lobe dysfunction, with impulsive distractable behaviour, apathy and loss of insight. Seizures occurred in 11 of 25 reported cases and in 8 of the present series, including Case 8 who was otherwise asymptomatic.

TABLE 5 PUBLISHED CASES OF NEUROACANTHOCYTOSIS

Reference	*Sex*	*Age (yrs) of onset*	*Age (yrs) at report*	*Consanguinity*	*Dementia*	*Seizures*	*Psychiatric features*	*Orofacial dyskinesia*	*Dysphagia*	*Dysarthria*	*Movement disorder*	*Muscle wasting*	*Muscle weakness*	*Tendon reflexes*	*SAPs*	*EMG denervation*	*CK (IU/l)*
Estes *et al* (1967)	M	27	50	O	+	+	+	+	O	+	CD	+	+	O	−	+	−
Critchley *et al* (1968)	M	23	29	O	+	+	O	+B	+	+V	CDT	O	O	O	N	O	N
Critchley *et al* (1970)	F	26	30	O	+	O	+	+B	+	+V	CDT	O	O	R	N	O	N
Aminoff (1972)	M	58	69	O	O	+	O	+B	+	+V	CT	+	+	O	R	+	236
	M	56	62	O	O	O	O	+B	+	+V	CT	+	+	O	−	+	32
Bird *et al* (1978)	M)	40	48*		O	+	+	+	+	+	CD	+	+	O	−	+	400
	F)	32	39*	+	O	O	+	+	+	+Co	C	O	O	O	−	−	−
	M)	14	31*		O	−	−	+	+	+	C	−	−	−	−	−	−
Kito *et al* (1980)	M	36	50	O	O	O	+	+	+	+V	CT	+	+	R	−	+	317
Sakai *et al*. (1981)	F)	29	38		O	+	−	+B	−	+	C	O	+	+	N	+	1000
	M)	28	31	+	O	O	−	+B	+	+	C	O	O	O	R	−	170
	M)	27	47		O	O	−	+B	−	+	C	+	O	O	−	+	378
	M	34	37	O	O	O	−	+B	−	+	C	+	O	O	−	O	131
Limos *et al* (1982)	M	28	32	−	+	O	+	+B	−	+	C	+	−	−	R	+	384
Yamamoto *et al* (1982)	M)	34	41	+	+	O	+	+B	−	+V	CDPT	+	+	O	−	+	261
	F)	34	40		+	+	+	+B	−	+	CPT	O	O	O	−	−	↑
Sotaniemi (1983)	F	24	29	+	O	O	−	+B	−	+V	CT	+	+	O	−	+	261
Gross *et al* (1985)	M)	36	46	O	−	+	−	+	+	+	CT	+	+	O	R	+	1136
	M)	40	51		−	+	−	−	−	+	CT	+	−	−	−	−	↑
Spitz *et al* (1985)	M)	13	46	+	O	O	−	+B	+	+V	PT	+	+	O	N	+	348
	M)	36	40		O	O	−	+B	+	+Co	PT	+	+	O	N	+	543
Serra *et al* (1986)	F)	33	38	+	O	+	+	+B	−	+	CDT	+	+	R	R	+	89
	M)	33	41		O	O	−	+	+	OV	CT	+	+	O	R	+	519
Sobue *et al* (1986)	M	40	57*	O	O	+	−	+	−	+	C	+	+	O	−	+	1704
Bharucha and Bharucha (1989)	F	42	50	O	O	O	+	+B	+	OV	CDT	O	O	R	−	O	N
Medalia *et al* (1989)	F	27	39	O	+	+	+	+B	−	+V	CT	O	+	R	N	O	278

* = dead,) = siblings, O = absent, R = reduced, + = present, − = not stated, N = normal, ↑ = elevated, B = tongue/lip biting, Co = coprolalia, C = limb chorea, D = dystonia, P = parkinsonism, V = vocalizations, T = tics, SAPs = sensory nerve action potentials, EMG = electromyography

All previous cases have had striking involuntary movements affecting the orofacial region, often sufficient to cause tongue and lip biting (17 of 25 cases) and to interfere with speech (24 of 26 cases) and swallowing (14 of 15 cases). Abnormal palatal and pharyngeal contraction may also contribute to the dysphagia (Critchley *et al.*, 1970). Many had vocalizations (11 of 26 cases), although coprolalia was only present in 2 (Bird *et al.*, 1978; Spitz *et al.*, 1985). In our own patients, dysarthria was also common, and was severe enough to cause virtual anarthria in some cases; involuntary vocalizations occurred in 9 cases. The orofacial movements usually were choreiform, but sometimes they appeared dystonic, or a mixture of both. Dystonic protrusion of the tongue induced by mastication in 2 of our patients has been clearly described in 2 other cases (Yamamoto *et al.*, 1982; Case 2, de Yebenes *et al.*, 1988). Orofacial dyskinesia was not always present, however, or biting of the tongue or buccal mucosa.

Dyskinesia affecting the limbs was described as choreiform in almost all reported cases, and broadly resembled that seen in Huntington's disease (HD). Likewise in our patients chorea dominated in 11 of the 17 with a movement disorder. However, the chorea tended to involve the legs earlier and with fewer movements of the forehead and hands than in HD. One member of family L, an obligate gene carrier aged 56 yrs (Case 6), only had subtle choreiform movements of the legs. Previous descriptions mentioned tics occasionally, and Shibasaki *et al.* (1982) claimed on the basis of electrophysiological studies that the mechanism responsible for the usual dyskinesias more closely resembled that responsible for voluntary movements. Amongst our own patients, repetitive stereotyped tic-like movements were evident in addition to chorea in 8 cases. Not all our cases had chorea, with or without tics. In 4 cases described here, dystonia was the presenting and dominant movement disorder throughout their illness; another 5 cases exhibited dystonia as well as chorea. Thus the dyskinesias occurring in neuroacanthocytosis have comprised chorea or tics or dystonia, or a mixture of these various abnormal movements.

Of interest is the observation in 2 brothers of progressive parkinsonism gradually replacing the hyperkinetic state (Spitz *et al.*, 1985) in the absence of neuroleptic drugs; the authors suggested that initial supersensitivity of central dopamine receptors is followed by neuronal degeneration. A similar course was observed by Sakai *et al.* (1985), and in our Case 2. In at least 5 other patients of ours, both hyperkinetic and akinetic-rigid features were present simultaneously, as also occurs in HD (Thompson *et al.*, 1988). Thus it seems that a complete range of extrapyramidal movement disorders, from tics and chorea through dystonia to parkinsonism, may occur in this condition. However, a movement disorder is not universal: 2 of our younger cases (Cases 8, 10) had neither dyskinesia nor parkinsonism, perhaps because they were observed early in their clinical course.

Amongst the more typical cases, cranial CT has often shown caudate atrophy (Serra *et al.*, 1987). In our own series this was noted only in the older patients with long duration of symptoms, and cerebral atrophy was more common. Of the 4 cases who had MRI, 3 also had focally altered signals from parts of the basal ganglia bilaterally that were nonspecific but consistent with increased tissue water content. Similarly situated lesions, although with different signal characteristics, were reported by Laplane *et al.* (1989) in patients following acute anoxic or toxic encephalopathy, all of whom exhibited obsessive-compulsive behaviour and a frontal lobe-like syndrome strikingly similar to that seen in some of the cases described here.

Most of the published cases had muscle wasting with weakness, areflexia, and modestly elevated serum CK activity. One case had coincidental cardiomyopathy (Faillace *et al.*, 1982), and was later reported to have the McLeod phenotype (*see below*).

Neurophysiology and peripheral nerve morphology

Motor nerve conduction studies in reported cases have always been normal. SAPs were reduced in 6 of the 11 cases for whom results were quoted and evidence of denervation was common on EMG and muscle biopsy. A similar pattern of neurophysiological abnormalities was confirmed in about half of our own cases. Visual, auditory and somatosensory evoked potentials were also normal in 2 cases reported by Kaplan *et al.* (1986) and in several of the patients described here.

Appearances suggestive of demyelination have been described in 5 of the 8 cases where peripheral nerve has been biopsied during life (Aminoff, 1972; Lantos and Aminoff, 1972; Ohnishi *et al.*, 1981; Serra *et al.*, 1986), but such changes could be secondary to axonal atrophy (Sobue *et al.*, 1986). Aminoff (1972) and Lantos and Aminoff (1972) reported 2 cases with nerve biopsies; on light microscopy both specimens were reported to show patchy demyelination, while 1 showed some evidence of axonal damage. EM in 1 patient showed the cytoplasm of several Schwann cells to contain unusually large dense bodies similar to secondary lysosomes. Swollen mitochondria and disrupted vesicles were seen in myelinated axons, and plate-like Schwann cell processes formed bands devoid of axons. In the other nerve biopsy reported, EM showed remnants of myelin lamellae in the cytoplasm of Schwann cells, several axons were said to contain polymorphic inclusions, and irregular mitochondria were frequently found in both myelinated and unmyelinated axons. Ohnishi *et al.* (1981) reported 3 cases who had nerve biopsies, of whom 2 showed a decrease in the density of large myelinated fibres. EM in 1 patient showed swelling of an unmyelinated axon filled with glycogen granules (beta particles).

In a quantitative study of a single case, Sobue *et al.* (1986) found severe depletion of anterior horn cells in a cervical segment, but not in thoracic or lumbar segments. Dorsal root ganglion cells were preserved. In cervical and lumbar ventral roots there was loss of myelinated fibres with many regenerative clusters. In dorsal roots, sciatic and sural nerves, there was selective loss of large diameter myelinated fibres. Anterior horn cell loss has been reported in 2 other cases but no details were given (de Yebenes *et al.*, 1988).

The pathology of the 3 sural nerves described here is indicative of a chronic axonal neuropathy with prominent regenerative activity. In the 2 biopsies the large diameter myelinated fibres were predominantly affected. The normal appearance of the anterior and posterior nerve roots at postmortem confirms that axonal degeneration occurred more distally; there was no obvious loss of dorsal root ganglion cells or of anterior horn cells in our autopsied case. The increased numbers of Schwann cell nuclei in the femoral nerve were probably due to axon regeneration, although teased fibre preparations showed less evidence of regeneration in this mixed nerve than in the sural nerve. An interesting feature was the occurrence of giant axonal changes in occasional fibres in 1 patient, the enlarged axons being filled with accumulations of neurofilaments. This suggests a disturbance of slow axonal transport in these fibres. However, in the postmortem nerve swollen axons were also associated with accumulations of a variety of organelles.

CNS neuropathology

In the 5 published cases, aged 36–48 yrs, where necropsy has been performed, marked neuronal loss and gliosis were seen affecting the caudate, to a lesser extent the putamen, and in 2 cases the pallidum (Bird *et al.*, 1978; Iwata *et al.*, 1984; Sobue *et al.*, 1986; de Yebenes *et al.*, 1988). No histological abnormality was found in any other region of the brain, including the substantia nigra. In contrast to the findings in HD, the cerebral cortex appeared to be spared and, in 1 case where postmortem neurochemical analysis was performed, activities of glutamic acid decarboxylase and choline acetyl transferase in cortex and striatum were normal (Bird *et al.*, 1978).

Depletion of dopamine and its metabolites in the neostriatum has been reported in 2 cases, together with elevation of noradrenaline levels in the putamen and pallidum, and reductions in the same regions of substance P and gamma-aminobutyric acid (GABA) concentrations corresponding to the degree of neuronal loss (de Yebenes *et al.*, 1988). The substantia nigra was also severely depleted of substance P, despite the absence of microscopic abnormality.

In our Case 2, the same pathological features of neuronal loss and gliosis were seen in the corpus striatum, pallidum, and also the lateral substantia nigra (pars reticulata), an area not previously reported to be affected. This may reflect the much older age at death (61 yrs) and longer duration of symptoms (22 yrs) in our patient, suggesting that the neostriatum is primarily and most severely affected, whereas its pallidonigral projection areas may be involved later in the course of the disease process. The cerebral cortex itself appeared spared. The extensive atheroma and several cerebral infarcts were presumably coincidental, since stroke-like episodes do not occur typically and there is no experimental evidence for rheological abnormalities.

Two brothers have been described with a similar clinical syndrome of amyotrophy with chorea involving orofacial tics and vocalizations in association not with acanthocytosis but with spherocytic haemolytic anaemia (Spencer *et al.*, 1987). As well as splenomegaly and cardiomyopathy, both had certain atypical neurological features such as pes cavus and glove and stocking sensory loss. Autopsy of the index case revealed neuronal loss affecting the striatum, substantia nigra and the anterior horns of the spinal cord, but striatal iron deposits and spheroid bodies were also present. Whether this sibship represents a true variant of the neuroacanthocytosis syndrome as the authors suggest seems doubtful.

McLeod phenotype

Kell is a complex blood group system with 21 serologically recognized erythrocyte antigens. The first recognized antigen K is highly immunogenic and thus Kell is the next most important blood group system after ABO and Rh in determining compatibility for transfusions. Weak expression of Kell system antigens together with the absence of the otherwise ubiquitous surface antigen Kx characterize the rare McLeod phenotype, first recognized and named after the propositus by Allen *et al.* (1961). About 25% of McLeod erythrocytes have acanthocytic morphology and affected subjects have a permanent compensated haemolytic state (Wimer *et al.*, 1977).

More than 60 examples of the McLeod phenotype have now been identified (Marsh and Redman, 1987), all of whom were males. The X-linked locus presumed to control Kx formation has been named XK. Because of a close association between X-linked chronic granulomatous disease and the McLeod phenotype, it was originally postulated that variant alleles at the same locus were associated with failure of production of the Kx surface antigen on erythrocytes and/or leucocytes (Marsh *et al.*, 1975). However, more recent studies have since identified 2 separate but closely linked loci in the Xp21 region, the XK locus lying between the Duchenne muscular dystrophy locus and that for chronic granulomatous disease (Baehner *et al.*, 1986; Bertelson *et al.*, 1988). It is also now known that Kx is absent from normal human granulocytes (Branch *et al.*, 1986). The XK locus is inactivated by the Lyon effect, and female carriers of the McLeod gene have mosaicism both in the Kell blood group system and in erythrocyte morphology, with a dual population of normal discocytes and anything between 5 and 95% acanthocytosis (Wimer *et al.*, 1977; Marsh and Redman, 1987; Bertelson *et al.*, 1988).

The extent of neurological involvement in the McLeod syndrome is unclear. Elevated serum CK levels of 1000–2500 IU/l, first reported by Marsh *et al.* (1981), are a constant feature, and an associated benign X-linked myopathy has been described in 2 asymptomatic men, who had no clinically detectable neuromuscular abnormality but raised CK levels and both EMG and histological evidence of a myopathy (Swash *et al.*, 1983). Marsh and colleagues (Marsh, 1983; Marsh and Redman, 1987) have reported that most affected males develop slowly progressive weakness and wasting of skeletal muscles, usually after the age of 40 yrs, with additional evidence of a cardiomyopathy, but no such serial observations have been published.

Great interest was aroused by the report of a male with a minor Xp21 chromosome deletion having clinical features of Duchenne muscular dystrophy, mental retardation, retinitis pigmentosa, chronic granulomatous disease and also the McLeod phenotype (Francke *et al.*, 1985). The apparent myopathy in McLeod males is, however, unlikely to be explained on the basis of an associated defect of the Duchenne gene itself, because the two loci are widely separated (Bertelson *et al.*, 1988). A possible alternative hypothesis of a common cell membrane defect affecting muscle and erythrocytes remains to be tested (*see* Hardie, 1989).

In one of the earliest reported kindreds with McLeod phenotype, the 5 affected males were originally described as healthy (Symmans *et al.*, 1979). However, subsequently Marsh (1983) reported that all except the youngest, aged 14 yrs, were areflexic and the oldest, aged 43 yrs, had 'choreiform movements of his extremities of steadily increasing severity, and mild dysarthria'. This patient and a second male are referred to in an abstract as having 'involuntary dystonic or choreiform movements, areflexia, wasting of limb muscles, elevated creatine kinase' (Schwartz *et al.*, 1982). Furthermore, the case reported by Faillace *et al.* (1982) as acanthocytosis and amyotrophic chorea presenting with cardiomyopathy was later shown to have McLeod phenotype, and 3 younger male relatives were found to have diminished or absent tendon reflexes and the same erythrocyte abnormalities (Marsh, 1983).

The 2 examples of McLeod phenotype found in family L were associated with acanthocytic morphology, elevated CK levels, areflexia and a movement disorder, although other members of family L with similar abnormalities had a normal Kell phenotype. One of the McLeod phenotype cases was remarkable because she was a female. The only other female thought to have this phenotype was the mother of a McLeod phenotype boy; however, analysis of her blood by fluorescent-activated cell cytometry revealed a dual population of McLeod (the majority) and normal Kell phenotype red cells (Carstairs *et al.*, 1988). These observations suggest an association between neuroacanthocytosis and the McLeod phenotype which is presumably genetic, although the McLeod phenotype was specifically excluded in at least some of the reported chorea-acanthocytosis subjects and other patients in the present series.

Genetics of neuroacanthocytosis

The genetics of neuroacanthocytosis are unclear; both autosomal dominant and autosomal recessive inheritance has been proposed. In favour of the former is parent-child transmission of acanthocytosis in a small number of families (Levine *et al.*, 1968; Kito *et al.*, 1980; Sotaniemi, 1983), but single generation familial cases or less certain pedigrees are more common. Sporadic cases are probably underreported. Parental consanguinity was present in 5 kindreds (Sakai *et al.*, 1981; Yamamoto *et al.*, 1982; Sotaniemi, 1983; Spitz *et al.*, 1985; Serra *et al.*, 1986), including a family with acanthocytosis but no neurological abnormality in 2 generations, perhaps representing pseudodominance with affected offspring from a heterozygote-homozygote mating (Sotaniemi, 1983).

Amongst other authors, Vance *et al.* (1987) concluded that neuroacanthocytosis is an autosomal recessive disorder. Family H, the affected sibling pair and the sporadic cases in this series would support this hypothesis. However, the existence of family L and others containing affected members in more than one generation requires either a different mode of inheritance or the possibility of genetic heterogeneity.

Analysis of previously published pedigrees and those presented here showed male to male transmission in only one, in which an affected male had a brother and a paternal uncle with seizures and 'mental symptoms', and a son with acanthocytosis alone without neurological impairment (Kito *et al.*, 1980). Of all familial cases, 31 were male and 18 female, and of sporadic cases 9 were male and 4 female (overall 40 male, 22 female). These data suggest the possibility of an X-linked gene causing neuroacanthocytosis, and the pedigree of family L could be explained on this basis, although autosomal dominant inheritance cannot be excluded. Apart from Case 5, the males in this kindred were more severely affected than the females, and there was no male to male transmission. Only 3 affected males in the present study have offspring. Screening the 3 children aged 11–25 yrs of Cases 7 and 13 has identified significant acanthocytosis in both females, including the daughter of Case 13 who is entirely normal and Case 10 who, unlike her brother, has minimal neurological abnormalities.

The association with the McLeod phenotype in Cases 5 and 9 also points to the existence of a locus for neuroacanthocytosis on the short arm of the X chromosome. It is of interest in this context that many patients with the McLeod phenotype have increased CK levels and that involuntary movements have been noted in some affected males. Molecular genetic analysis of the kindreds reported here using DNA probes mapping to the region of the McLeod locus is in progress.

The mechanism of acanthocytosis

The unique morphology of acanthocytes has generally been attributed to a membrane lipid defect; in abetalipoproteinaemia to an increase in sphingomyelin content and the sphingomyelin/lecithin molar ratio, and in the 'haemolytic spur-cell anaemia' of liver disease to a marked increase in membrane cholesterol and an elevated cholesterol/phospholipid molar ratio (Cooper *et al.*, 1975; Owen *et al.*, 1982, 1985). Such a mechanism seems unlikely to explain the acanthocytosis seen in our patients, since no overt defect in erythrocyte membrane lipid composition could be found. This confirms results of limited studies in other cases (Estes *et al.*, 1967; Aminoff, 1972; Villegas *et al.*, 1987), although a small reduction in linoleic acid content was reported in 1 propositus (Bird *et al.*, 1978) and an increase in the sphingomyelin/phospholipid ratio in another (Clark *et al.*, 1989). Increases in membrane fluidity were induced at much lower concentrations of adenosine diphosphate than those normally required in one report (Betts *et al.*, 1970). A serum factor has been postulated by some authors (Estes *et al.*, 1967; Aminoff, 1972) but not confirmed by us or by others (Betts *et al.*, 1970; Clark *et al.*, 1989).

One study with freeze-fracture EM of erythrocyte membranes reported a significant increase in areas lacking intramembranous particles (Ueno *et al.*, 1982), a novel observation suggesting an abnormality of membrane fluidity, but this was not confirmed by Clark *et al.* (1989). In the absence of any major differences in lipid constitution, conformational defects of the intrinsic membrane proteins may well be relevant (Asano *et al.*, 1985). The mechanism of acanthocytosis in the McLeod phenotype itself is equally uncertain but is presumably related to the primary abnormality of membrane proteins exposed on the extracytoplasmic surface (*see* Hardie, 1989).

Diagnosis of neuroacanthocytosis

The typical clinical picture of neuroacanthocytosis comprising a movement disorder, personality change and progressive intellectual deterioration most closely resembles that of HD, which is not surprising in view of the marked similarities on neuropathological examination. In view of important differences in implications for genetic counselling, all suspected cases of HD should be screened for acanthocytosis, particularly if there are any unusual features such as seizures, vocalizations, biting of lips or tongue, amyotrophy, areflexia, or a negative family history. Acanthocytosis should also be sought in atypical cases of Tourette syndrome, dystonia, seizures or peripheral neuropathy.

New cases of neuroacanthocytosis will only be accurately diagnosed if there is a close liaison between the clinical neurologist and the haematology laboratory. Most laboratories are now placing increased reliance on automated analysers, so that blood films are only examined if the red cell parameters, platelet count or leucocyte differential counts are found to be abnormal. It is therefore essential that the laboratory is notified if the condition is suspected clinically, to ensure that the blood films are made promptly following receipt of the sample and are carefully examined.

Laboratory staff must be adequately trained to evaluate erythrocyte morphology and distinguish true pathological acanthocytes from echinocytes and other potential artefacts induced during preparation of smears. Once erythrocyte abnormalities have been identified, accurate quantitation of the percentage of abnormal forms is helpful, although we are uncertain whether this necessarily correlates with severity of neurological impairment.

Dried blood smears from 16 of 32 maternal relatives examined by standard light microscopy by Levine *et al.* (1968) were said to reveal acanthocytosis. No distinction from echinocytes was made and they regarded more than 1% as significant. However, this figure should be treated with caution since, in the haematological paper (Estes *et al.*, 1967) the authors consider 6 cousins with 1.2–6.2% acanthocytosis as significant despite the transient finding of 4.5% typical acanthocytes in 1 control subject and 5–13% 'moderately crenated red cells' in 3 other controls. According to Dacie (1985), adults may normally have up to 3% echinocytosis, and additional factors may increase this (*see* Hardie, 1989). We therefore chose a more stringent level of significance at >3% true acanthocytosis and, since echinocytes and acanthocytes invariably coexist, the total proportion of all crenated forms was always higher than this.

Some of our cases (Cases 2, 9, 14, 15, 16) were only found to have significant degrees of acanthocytosis on repeated sampling. This reflects not only an increasing awareness of the condition amongst laboratory staff, but also the fact that the numbers of echinocytes and acanthocytes may at times be relatively few, especially early in the clinical course. All the living cases we have described had significant acanthocytosis as defined above, and the numbers of acanthocytes have either persisted or increased since the time of diagnosis. However, we wish to emphasize that, when there is sufficient clinical suspicion of the disorder, one negative blood film does not exclude the diagnosis. Repeated blood samples should be examined at intervals.

SEM remains a most helpful adjunct in assessing the variation and extent of the erythrocyte morphological abnormalities. The technique described here is straightforward, and accurate quantitation can be obtained by examining several fields. If blood is collected directly into 1% glutaraldehyde solution, artefactual morphological changes induced by prolonged exposure to EDTA are avoided and thus the SEM appearances provide a useful control for light microscopy of conventional blood smears. We found no instance of discrepancy between the two techniques, and in some cases the abnormalities were more evident on SEM, which may therefore be particularly valuable when light microscopic appearances are of borderline significance.

Neurologically asymptomatic relatives with significant acanthocytosis have also been identified in the course of family screening by a number of authors, and in the only child of our Case 13. However, apart from our own Case 10 referred to by Lowe *et al.* (1977), we are not aware of any follow-up observations and it is not known whether such people will inevitably develop the full clinical syndrome or whether some may remain entirely free of neurological disease. Prospective studies of subjects both clinically affected and those at risk will be necessary to study the evolution of the haematological and clinical abnormalities.

ACKNOWLEDGEMENTS

We are grateful to Drs G. Boddie, A. J. Lees, J A. Morgan-Hughes, W. Michael, C. H. Hawkes, S. D. Shorvon, K. Sivakumar, P J. B. Tilley, T. J. Fowler and Professors D. L. McLellan and D. C. Taylor for referring cases and allowing us to report cases under their care.

We wish to thank C. Gilois, A. Lai and the staff of the Haematology Laboratory, National Hospital, and also R. Senkus and K. Davies of the Electron Microscope Unit of King's College Hospital Medical School for processing the blood samples; Dr J. Delhanty for performing karyotype analysis; and Dr T. W. Robbins and members of Professor E. K. Warrington's department for carrying out neuropsychological testing of many of the patients. Y. Imai was a recipient of a Wellcome-Japanese Fellowship, and V. P. Misra was supported by Efamol Ltd. Financial support from the Brain Research Trust is also gratefully acknowledged.

Parts of this work have been communicated to meetings of the Association of British Neurologists, the British Neuropathological Association and the Peripheral Nerve Study Group.

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(*Received October 24, 1989. Revised December 28, 1989 Accepted January 3, 1990*)

APPENDIX

Case reports

Family H (fig. 1)

Case 1 (II 1, male, died aged 61 yrs). Difficulty walking and poor balance were first noted at the age of 37 yrs He was admitted to a psychiatric institution aged 42 yrs because of a progressive personality change characterized by emotional lability and antisocial behaviour, when he was found to be dysarthric with dysphagia and areflexia. There was a slow but progressive deterioration in his speech and walking over the next 18 yrs, and he was never discharged from hospital, Infrequent generalized seizures began in 1980.

When seen at the National Hospital in 1982, 9 months before his death aged 61 yrs, speech was limited to grunts and he communicated by writing He was thought to be mildly demented. Upgaze was limited and voluntary eyelid opening was difficult. Movements of the face, palate and tongue were reduced and slow with drooling of saliva, brisk pout and jaw reflexes and marked axial rigidity Muscle tone in the limbs was slightly increased, with severe bradykinesia, normal coordination, mild distal weakness in the lower limbs, areflexia and bilateral extensor plantar responses There was clawing of the toes, equinovarus deformity of the feet and his gait was shuffling. Sensory examination was normal

A CT brain scan showed mild cerebral atrophy and flattening of the caudate nuclei Nerve conduction studies (NCS) demonstrated small SAPs and normal MNCVs in the ulnar and median nerves. The small foot muscles were denervated. Leucocyte enzymes and EM of a skin biopsy were normal. Acanthocytes were not seen in a dried smear of peripheral blood. An autopsy was not performed.

Case 2 (II.3, female, died aged 61 yrs). Abnormal movements began around the mouth when aged 39 yrs, producing difficulty with speech and swallowing, when taking no medication Examination at the National Hospital in 1969 when aged 42 yrs showed dysarthria with frequent orofacial movements, teeth grinding, tongue protrusion and spitting. Full scale IQ was 86, suggestive of mild intellectual impairment Over the next 8 yrs generalized chorea developed in the trunk and limbs and she became increasingly agitated with emotional lability, but the chorea gradually subsided over the next 4 yrs.

By the time of her admission in 1981 at the age of 54, swallowing was impaired and she had lost weight Psychometric assessment indicated further global cognitive impairment. Pursuit eye movements were saccadic and upgaze was limited Dysarthria was severe, with spastic weakness of the face, palate and tongue and drooling of saliva Her posture was flexed and unstable, with rigidity and marked bradykinesia of the limbs and distal muscle wasting. Reflexes were present in the arms, absent in the legs, and both plantar responses were extensor

CT brain scan showed caudate atrophy as well as some central cerebral atrophy. A routine blood film was reported to show moderate anisopoikilocytosis with fragmented and crenated cells, attributed at the time to a mild proven iron-deficient anaemia (Hb 10 1 g/dl) related to poor diet. NCS were not performed. Her disability progressed and she died in 1988

Case 3 (II.8, female, aged 49 yrs). When admitted to the National Hospital in 1982 aged 44 yrs, there was a 4 yr history of dragging the left leg, micrographia and slurred speech, fidgety movements, and mild intellectual impairment. Verbal and performance IQs were 94 and 96. Slow jerky pursuit eye movements, facial impassivity, slurring dysarthria and tongue tremor were noted. Limb tone was normal but there was bilateral rest tremor and mild bradykinesia of the upper limbs, rather variable jerky choreiform movements proximally in all four limbs and focal action dystonia of the left leg. Tendon reflexes were all diminished and the ankle jerks absent. Plantar responses were flexor. Occasional 'burr cells' were seen in a peripheral blood smear but haematological parameters were normal.

Two years later she developed frequent distressing involuntary belching. There was a mild iron-deficient anaemia, Hb 10.5 g/dl, with a microcytic hypochromic picture and 'occasional burr cells'. Treatment with sulpiride was commenced for a presumed diaphragmatic tic. She was readmitted 1 yr later when belching interfered with speech, swallowing and sleep. She had become more bradykinetic with mild extrapyramidal rigidity in the limbs. Treatment with levodopa/benserazide was substituted for sulpiride. She had also developed bilateral 40 dB deafness with mixed conductive and sensorineural features, and required a hearing aid. Routine microscopy of her peripheral blood was reported to show 'moderate anisopoikilocytosis, frequent burr cells and elliptocytes', with Hb 11.1 g/dl.

Five years after the initial presentation, belching persisted and she had begun to fall occasionally. Psychometric assessment suggested evidence of mild cognitive deterioration (verbal/performance IQ 88/91). There was a supranuclear disturbance of eye movements, with poor upward gaze and convergence, intermittent blepharospasm, and dystonic dysarthria with marked hesitancy and dysprosody of speech. Tongue movements were slow and tremulous. There was hyporeflexia with extrapyramidal rigidity and bradykinesia in the limbs as well as occasional chorea. Gait was mildly parkinsonian, with impaired postural reflexes. Sensation was normal.

Copper studies, CSF, NCS, somatosensory evoked potentials (SEPs), biopsies of bone marrow and rectum, autonomic function tests, and CT brain scan were normal. MRI of the brain showed nonspecific high signal from the periventricular white matter with mild dilatation of the lateral ventricles and slight caudate atrophy. Although a mild microcytosis had been documented previously, most recent Hb was 10.8 g/dl with normal MCV 83.4 fl, and about 20% abnormal erythrocytes, both echinocytes and acanthocytes. Serum CK was elevated at 401 IU/l.

Case 4 (II.9, female, aged 47 yrs). Depressive symptoms and marked obsessional features including checking behaviour developed at the age of 44 yrs. Neurological examination then was normal, but thereafter she gradually became more forgetful, with slurred speech and later, sudden jerking movements of the limbs and trunk which threw her off balance and occasionally caused falls. She had been treated with a variety of antidepressant and neuroleptic drugs over the previous 2 yrs, and was taking tetrabenazine clomipramine and promazine on admission to the National Hospital in 1988, aged 47 yrs.

On examination she was emotionally labile and fatuous with verbal perseveration. There was evidence of organic personality change with primarily obsessional and frontal lobe features. Psychometric assessment estimated her verbal and performance IQ at 87 and 89, in the dull average range rather below her estimated premorbid level.

She had a slurring dysarthria and mild bilateral deafness. There were no orofacial involuntary movements, although infrequent belching was noted. There was mild rest tremor, moderate extrapyramidal rigidity and severe bradykinesia in the left arm and similar, but less marked, features on the right. There was mild dystonia in the left leg with bilateral infrequent choreiform kicking movements at the hips and knees. Tendon reflexes were all absent, plantar responses flexor. Vibration sense was reduced in the feet. Gait was normal although arm swing was absent bilaterally.

Full blood count was normal, with 25% acanthocytosis by light microscopy. Visual, auditory and sensory evoked potentials were all normal. NCS suggested a mild axonal peripheral neuropathy, with small SAPs, normal MNCV, and evidence of denervation on EMG of the intrinsic hand muscles. Bone marrow examination and CT brain scan were normal.

Other members Case I.2 died at the age of 63 yrs from stomach cancer and was said to be neurologically normal. Case I.1 was deaf from an early age, dysarthric, and mildly mentally subnormal. He was an inpatient in a geriatric hospital from the age of 60 until his death from lung cancer aged 67 yrs. His dysarthria was thought to be related to deafness and the family do not think he was affected. No autopsy was performed. All other individuals in this pedigree indicated as normal and asterisked (fig 1) were neurologically normal and had normal blood films.

Family L (fig. 1)

Case 5 (II.1, female, aged 57 yrs). When admitted to the National Hospital following referral of her grandchildren for genetic counselling, she gave a 6 yr history of diabetes and infrequent generalized tonic-clonic seizures for which she was taking metformin and phenytoin, respectively. She denied other neurological symptoms but her relatives had noticed that she was fidgety and rather vague.

On examination she had a slurring dysarthria, was discursive and distractable, and her conversation was repetitive and trivial in content. There was no oral self-mutilation but she was generally unkempt with bitten fingernails and there were sores on her fingers. Her gait was lurching and ataxic. She had generalized chorea, chiefly affecting the lower trunk and legs, and occasional tic-like facial movements. The tendon reflexes were absent and both plantar responses were extensor. On a computerized neuropsychological test battery, her performance was particularly impaired on tests of attentional shifting, working memory and planning, thought to be sensitive to frontal lobe dysfunction.

Over half of all erythrocytes were abnormally crenated with almost equal proportions of echinocytes and acanthocytes, and serum CK was elevated at 292 and 840 IU/l on consecutive occasions. Fasting glucose was 10 mmol/l. EEG showed generalized paroxysms of irregular slow waves. Median, ulnar and sural SAPs were undetectable; ulnar and tibial MNCV were normal. EMG of distal muscles showed evidence of denervation. CT brain scan showed mild generalized cerebral atrophy and more marked focal atrophy of the caudate nuclei, and 2 small low density lesions in the deep white matter adjacent to the frontal horns.

Case 6 (II.2, female, 56 yrs). There were no neurological symptoms On examination, higher intellectual function was normal. There were occasional choreiform movements of the feet. The rest of the neurological examination, including the tendon reflexes, was normal. About 20% acanthocytosis was present and serum CK was slightly elevated at 265 IU/l.

Case 7 (III.1, male, died aged 31 yrs). This man, previously reported by Lowe *et al.* (1977), was well until the age of 24 yrs when he had a generalized tonic-clonic seizure. On admission to hospital he was febrile and postictal; the tendon reflexes were absent. CSF examination was normal but serology suggested a recent influenza B infection and a diagnosis of viral encephalitis was made Blood film showed numerous acanthocytes, and serum CK was persistently elevated (600 IU/l several weeks after the seizure) Serial EEGs showed paroxysmal generalized slow wave activity, and he was treated with phenytoin. He had a further 2 seizures 8 months later, when acanthocytes and elevated CK were again present. CT brain scan was normal Median and ulnar SAPs were undetectable, MNCV and EMG were normal

Over the next 6 yrs his personality changed but he was able to work as a policeman. Towards the end of his life he was noted to be fidgety, had poor balance, and tended to flap his feet on walking. He was found dead in bed in 1982 at the age of 31 yrs. Postmortem examination showed left ventricular hypertrophy, hepatic congestion and cerebral oedema; histological examination was not performed.

Case 8 (III 3, male, died aged 31 yrs). Examination at the age of 20 yrs, in relation to his brother's illness, showed areflexia. Serum CK was elevated at 1980 IU/l and there was acanthocytosis (Lowe *et al* , 1977), but he remained without symptoms until 2 yrs later when he had his first generalized seizure. CT brain scan was normal and EEG showed generalized slow wave discharges Despite anticonvulsant therapy he continued to have seizures approximately every month but was apparently otherwise well He died in 1987 at the age of 31 yrs, probably during a seizure Autopsy showed marked left ventricular hypertrophy, hepatomegaly with fatty change, and 'gross injection' of the brain; histological examination was not performed.

Case 9 (III.4, male, 27 yrs). There were no neurological symptoms but on examination he had mild generalized chorea and multiple tics All tendon reflexes were absent. Serum CK was elevated at 4035 IU/l and there was 10% acanthocytosis.

Case 10 (IV.1, female, 13 yrs). This girl developed normally and had no neurological symptoms. On examination the ankle jerks were depressed but there were no involuntary movements. EEG showed an excess of background slow activity and occasional generalized slow wave discharges. Serum CK, sural SAP and median MNCV were normal Mild acanthocytosis was present aged 3 yrs (Lowe *et al.*, 1977) and also (about 5%) when examined recently

Other members. Case I.3 died at the age of 55 yrs and had nocturnal seizures from the age of about 40 yrs His wife, I.4, is alive and well in her eighties. No details are known of his brothers, I.1 and I.2 Their mother died in the eighth decade of life and was said to have had no neurological problems; the family had no knowledge of their father. III.2, now aged 34 yrs, was not examined, but had previously been found to have normal tendon reflexes (Lowe *et al.*, 1977). She is currently asymptomatic, with normal CK and erythrocyte morphology All other members of this pedigree indicated as normal and asterisked (fig. 1) were neurologically normal aged between 49 and 8 yrs and did not have acanthocytosis. Serum CK was marginally elevated in 2 males, III.6 and III.8, both aged 25 yrs, but normal in all others (Table 3)

Family B

Case 11 (female, 24 yrs) Between the ages of 5 and 12 yrs, this girl developed behavioural problems with temper tantrums, school phobia and refusal Facial grimacing, eye winking and head shaking tics were first noted aged 12 yrs, together with ritualistic touching and kissing of objects. She left school aged 16 with educational attainments in the average range. Thereafter there was a slow progressive disturbance in her speech, which became slurred, festinant and stuttering, and also in her gait, with increasingly frequent freezing of gait and falls

She never worked and, by age 19 yrs, had become unable to walk outdoors without assistance Her behaviour was child-like. She was investigated at that stage without a diagnosis being made, although acanthocytosis was specifically sought and not confirmed She was referred back at age 24 yrs because of further progression in her behaviour, speech and gait disturbance.

On examination, her behaviour was immature, with features of an obsessive-compulsive disorder, with repetitive touching and kissing of her hands, light switches and other objects She exhibited limited spontaneous conversation and emotional lability Verbal and performance IQ was estimated at 81 and 106, respectively, below her estimated average premorbid level and with inefficiency on frontal lobe tasks Her smile was dystonic, with mild orofacial tics but no lip biting. Speech was hypophonic with marked dystonic dysarthria, dysprosody and festination. Tongue movements were slow Examination of the limbs was normal apart from mild bradykinesia, action dystonia and occasional motor tics. Tendon reflexes were preserved with flexor plantar responses and normal sensation She walked with small steps and frequent freezing of gait Postural reflexes were markedly impaired, with episodes of either festination or retropulsion

Peripheral blood contained about 30% echinocytes and 15% acanthocytes. Routine biochemical tests including copper studies and serum CK were normal SAPs were of reduced amplitude but MNCV, EMG, EEG and CT brain scan were all normal

Case 12 (male, 23 yrs) The brother of Case 11 was diagnosed whilst screening the family, although the parents had suspected for some years that he was suffering from the same condition as his sister. They first became concerned about his reading and poor school performance at age 8 yrs. After repeated assessments by psychologists, he was eventually diagnosed as dyslexic aged 14 yrs He was said to have poor visuospatial skills, writing and spelling, his reading was slow and his educational attainments were below average when he left school aged 16 yrs. Over the next 7 yrs his speech became progressively unintelligible and his behaviour childish with emotional lability and poor concentration. He never worked and, apart from the speech disturbance, was asymptomatic, although he had had a number of unexplained falls

On examination he was extremely distractable with disinhibited and child-like behaviour. Speech was hypophonic with marked dystonic dysarthria, dysprosody and festination Tongue movements and jaw reflex were normal. Examination of the limbs was normal apart from mild action dystonia. Postural reflexes were slightly impaired but tendon reflexes and gait were normal Peripheral blood contained about 10% echinocytes and 15% acanthocytes. Serum CK was normal at 103 IU/l.

Other members Both parents have been examined by us and are normal, with normal serum CK and no acanthocytosis by light microscopy and SEM of peripheral blood. The father has a brother and sister in their fifties who are said to be well, the mother is an only child. All 4 grandparents lived into their eighties without any neurological illness.

Sporadic cases

Case 13 (male, 51 yrs) There was a history of hospital admission for depression aged 26 yrs, treated with electroconvulsive therapy. Although his mood returned to normal, his wife noted an insidious personality change with the development of antisocial habits and irritability He developed progressive generalized chorea from the age of 44 yrs when on no drug treatment, followed by intermittent choking episodes. Because of increasing behaviour disorder, he was admitted to a psychiatric hospital aged 47 yrs and remains resident there. He had 2 generalized tonic-clonic seizures aged 50 and 51 yrs.

On examination aged 51 yrs, his behaviour was very apathetic and his verbal and performance IQs were 73 and 76, below his estimated average level. There was mild impairment of saccadic and smooth pursuit eye movements, facial impassivity with mild orofacial dyskinesia, occasional blepharospasm and involuntary vocalizations. Tongue movements were very slow, with sialorrhoea and a brisk jaw reflex. Chorea was mild and generalized (on sulpiride and tetrabenazine) with additional and severe parkinsonism, and a lurching gait. The small muscles of the hands and feet were wasted, tendon reflexes were absent, plantar responses were flexor and vibration sense was impaired distally. The parkinsonism had not resolved 3 months after withdrawal of medication. About 30% acanthocytosis was present in peripheral blood and serum CK was slightly elevated at 261 IU/l EEG, SAPs and MNCV were all normal, but MAPs were small and there was evidence of denervation on EMG CT scan showed mild cerebral and caudate atrophy.

He has 2 sisters aged 60 and 58 yrs. The younger one has had several admissions to a psychiatric hospital for psychotic depression since the age of 30 yrs, although her symptoms are currently controlled on treatment with thioridazine Both sisters have been examined by us and are neurologically normal, with normal serum CK and no significant acanthocytosis. He also has a 25-yr-old daughter who is asymptomatic and normal on neurological examination with normal serum CK activity, although 2–3% echinocytosis was present on dried blood smears and a few acanthocytes were present However, examination of wet blood films and SEM both confirmed the presence of significant (3–4%) acanthocytosis

Case 14 (male, 67 yrs) Mild chorea first developed at 62 yrs following a febrile illness and rash, when a provisional diagnosis of Sydenham's chorea was made despite negative antistreptolysin antibodies. His personality gradually changed and he became apathetic. The involuntary movements were reduced partially by tetrabenazine treatment but later increased, and he was noted to be clumsy with slurred speech About 1 yr before admission to the National Hospital, he developed loud involuntary vocalizations.

On examination, he was vague and distractable. There was a marked discrepancy between performance (101) and verbal (123) IQs, with impairment of memory, motor apraxia, general intellectual inefficiency and features suggestive of mild frontal 'subcortical' dementia. There was ocular impersistence with normal pursuit and saccadic eye movements and evidence of a gaze apraxia to the right with head-eye dyssynergia. His speech was slurred, with mild orofaciolingual dyskinesia, facial tics and repetitive grunts, sniffs and nonverbal vocalizations. There were brisk pout and grasp reflexes and coexistent but mild parkinsonism and distal choreiform and dsytonic limb movements brought on during walking or other motor activity. Tendon reflexes, plantar responses and sensation were normal His gait was shuffling with absent arm swing and hesitation with halting every 3–4 strides.

About 10% acanthocytosis was present. Serum CK was normal. EEG showed mild slowing, NCS and EMG were normal CT brain scan demonstrated mild cerebral and cerebellar atrophy with some white matter low density changes and slight flattening of the caudate nuclei. MRI showed scattered areas of altered signal from the deep white matter, and slightly increased signal (increased T_2) from the putamen bilaterally on spin echo sequences.

Case 15 (female, 25 yrs). At the age of 22 yrs, this woman (also reported by Meierkord and Shorvon, 1990) had 2 generalized seizures. She continued to have clusters of 2–8 seizures in a day at intervals of several months despite anticonvulsant therapy Fidgety movements were first noticed aged 23, and she became forgetful and developed slurred speech.

When aged 25 yrs her verbal and performance IQs were 92 and 95, having been 100 3 yrs earlier and below her estimated bright average level. Her speech was slurred with marked dysprosody. On examination, there were frequent motor stereotypies and tics, consisting of sucking, grunting and sniffing noises, chewing and grimacing, repetitive blinking, shoulder shrugging and trunk twisting, and choreiform movements of the fingers and toes. Muscle bulk and tendon reflexes were normal, with flexor plantar responses and normal sensation. There was 25% echinocytosis and 10% acanthocytosis. EEGs showed focal bitemporal slow wave and occasional irregular spike and wave activity. NCS and EMG were normal. CT brain scan showed mild cerebral atrophy and MRI showed symmetrical areas of altered signal (increased T_2) from the caudate bilaterally on spin echo sequences, and reduced signal from the putamen on T_1 weighted sequences

One year later, there had been a marked personality change with increasing anxiety, emotional lability and carelessness. She had also started to complain that her tongue tended to push food out of her mouth when trying to eat, and that she occasionally bit her lips or tongue inadvertently Serum CK had risen from 860 to 1635 IU/l. The involuntary movements were helped partially by sulpiride treatment.

Both parents have been examined by us, and were neurologically normal with no acanthocytosis. She has 1 brother who is said to be well.

Case 16 (female, 36 yrs). On referral when aged 33 yrs there was a 5 yr history of generalized tonic-clonic seizures, usually occurring in clusters of 6–12 seizures over about 36 h at intervals of about 3 months and often at the time of menstruation. When aged 29 yrs she developed involuntary shoulder shrugging and head jerking, gradually became more clumsy and was said to be anxious Constant chewing movements had caused her to bite her tongue or the inside of her cheeks, and she kept a piece of cloth in her mouth to protect the mucosa

On examination, she was impulsive and distractable There were continuous orofacial movements and mild generalized chorea. EEG showed a slow dominant rhythm with mild bitemporal abnormalities, but CT brain, EMG and NCS were normal. Muscle biopsy showed no evidence of a storage disorder. CK was slightly elevated but no acanthocytes were seen on a wet blood film at that time

She had no further seizures on anticonvulsant medication, but her home became increasingly untidy and she became less sociable, with slurred speech and difficulty eating because of tongue protrusion. When reviewed at the age of 36 yrs, there was evidence of some cognitive decline, with a frontal affect, slurring dysarthria, infrequent vocalizations, tongue and lip biting with lingual dystonia induced by eating, moderate limb chorea with motor tics and hyporeflexia

Acanthocytes (about 10%) were present repeatedly in routine blood films on this occasion. Both parents have been examined by us, and are neurologically normal with no acanthocytosis. She has 1 sister and 1 brother who are said to be well

Case 17 (male, aged 18 yrs). This boy presented with an 8 yr history of slowly progressive generalized dystonia The only child of healthy unrelated parents, his birth and developmental milestones were normal At age 5 yrs he was diagnosed as having dyslexia When aged 8, dystonic posturing of the right hand was first noticed while attempting to pick up objects Hand movements became increasingly impaired by dystonia. From the age of 10 yrs, he attended a special school because of learning difficulties. When first seen by a neurologist at age 14, there was mild generalized limb dystonia and treatment with benzhexol was initiated

When seen at the National Hospital 2 yrs later, he was able to dress and feed himself with difficulty. His speech had become slurred and indistinct He could run, although with a recent tendency to trip and fall. His height, weight and sexual development were all below the third centile, but general physical examination was otherwise normal. Psychometric assessment showed borderline function on both verbal and nonverbal tests of general ability, with verbal and performance IQs of 79 and 75 respectively. The only neurological abnormality was intermittent generalized 'overflow' dystonia of moderate severity, precipitated by limb movements and affecting facial muscles, speech and all four limbs. Tendon reflexes and plantar responses were normal, and he was able to walk and run with only minor impairment.

Microscopy of peripheral blood smears revealed about 8% abnormal crenated red cells, half of which were acanthocytes. Routine biochemistry, serum CK and copper studies, NCS and EMG were normal CT brain scan was normal but MRI showed focal abnormal signal (increased T_2) from the pallidum bilaterally on spin echo sequences consistent with increased tissue water content (fig. 3)

When reviewed 2 yrs later, he had become almost completely anarthric and was just able to vocalize single words with effort. His food had to be liquidized because of increasing dysphagia. Limb dystonia was more severe with a significant faster choreiform element, particularly affecting the right arm which was functionally useless. He was still able to walk normally. There was some improvement in motor function on a combination of anticholinergic and levodopa treatment A slight increase in morphological erythrocyte abnormalities was seen in serial samples to 5% echinocytes and 10% acanthocytes.

Both parents and his only brother were examined by us and found to be neurologically normal with no acanthocytosis.

Case 18 (male, 36 yrs) There was a 3 yr history of progressive speech impairment, dysphagia, intermittent muscle spasms affecting the left-sided limbs, and difficulty walking with occasional falls. The main abnormalities on neurological examination were a fine slow tremor of the tongue at rest and action dystonia involving the tongue and the left side of the face during speech, which was obviously dysarthric. He also spontaneously made intermittent loud sucking and blowing noises. The optic fundi and eye movements were normal. Tone and power in the limbs were normal, but rapid finger movements were mildly impaired in the left hand and dystonia of the left lower limb developed when walking.

Tendon reflexes were all reduced but present with reinforcement. The plantar responses were flexor. About 2% echinocytosis and 5% acanthocytosis was present in peripheral blood and serum CK was elevated at 692 IU/l. MNCV and EMG were normal but SAPs were of reduced amplitude. CSF examination, EEG and CT brain scan were normal

Case 19 (male, 44 yrs) His health had been good until, at age 39 yrs, he developed intermittent slurring of speech, dysphagia and dragging of the left leg while walking His disabilities progressed rapidly, and at age 40 a brain biopsy had been performed which was normal He developed occasional seizures following craniotomy, controlled by carbamazepine and clonazepam, but had taken no other medication. By the time of presentation, only occasional spoken words were intelligible and he required a communication aid. Walking had also become increasingly impaired because of action dystonia.

On examination, there was no intellectual impairment. Optic fundi and eye movements were normal There was a severe dystonic dysarthria with slowness of tongue movement. He spontaneously made occasional gasping and sighing noises Limb power and coordination were normal but there was dystonia at rest, more evident on action, in the left more than right-sided limbs, and severe generalized axial and limb action dystonia when standing or walking, causing marked postural instability with a tendency to lurch forwards. Tendon reflexes were sluggish in the upper limbs and absent in the lower limbs, with flexor plantar responses and normal sensation

About 10% echinocytes and 25% acanthocytes were present in peripheral blood and serum CK was elevated at 1980 IU/l. All other biochemical investigations including copper studies and vitamin E were normal. Biopsies of bone marrow and skin were also normal. NCS showed a mild axonal polyneuropathy in the lower limbs CT brain scan showed signs of the right frontal biopsy and mild cerebral atrophy.

ADDENDUM

Since the manuscript was submitted, several more cases of neuroacanthocytosis have been described Postmortem neuropathological findings in 2 brothers were reported by Alonso *et al.* (1989), whilst Vita *et al.* (1989) have extended the observations on peripheral neuropathy first made by Serra *et al* (1986). Positron emission tomography in a few cases has provided in vivo evidence of reduced glucose metabolism (Dubinsky *et al.*, 1989) and dopamine function (Brooks *et al.*, 1990, 1991, Peppard *et al* , 1990) affecting the striatum

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THE STROKE SYNDROME OF STRIATOCAPSULAR INFARCTION

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SUMMARY

Striatocapsular infarction has recently been described as a distinct stroke entity and forms an important subgroup of subcortical infarctions. In a prospective study of 50 consecutive patients over a 10 yr period with this syndrome, clinical and neuropsychological features, pathogenesis and outcome were studied to provide information concerning management and prognosis The most common clinical presentation was that of a stroke affecting mainly the upper limb with cortical signs such as dysphasia, neglect or dyspraxia. Evidence from EEG, angiographic and neuropsychological data supported a vascular/haemodynamic basis for the presence of the acute neuropsychological changes, while the chronic changes were more likely to be due to diaschisis. A study of risk factors and cerebral angiography enabled 4 pathophysiological subgroups to be identified: (1) cardiac emboli to the origin of the middle cerebral artery; (2) severe extracranial carotid artery occlusive disease with presumed embolism to the same site and/or involvement of haemodynamic factors; (3) proximal middle cerebral artery abnormalities causing occlusion of multiple lateral striate arteries at their origins; (4) normal angiography where pathogenesis was uncertain. The risk factors of cardiac disease and smoking were significantly increased as compared with age and sex-matched controls with other forms of ischaemic stroke. Stroke or vascular death rate was 2.7% per yr during a mean follow-up period of 2.25 yrs. Predictors of an excellent recovery with return to normal lifestyle were younger age, only brachial or brachiofacial weakness with absence of cortical signs at presentation and minimal change on angiography. This stroke entity deserves particular recognition in the spectrum of subcortical infarctions because of its specific pathogenesis, distinct neuropsychological features and reasonable prognosis.

INTRODUCTION

Striatocapsular infarction involves the territory of the lateral striate branches of the middle cerebral artery with sparing of the overlying cortex (Bladin and Berkovic, 1984). The clinical syndrome is usually one of a hemiparesis, affecting mainly the upper limb, in association with cortical abnormalities such as aphasia, neglect and/or apraxia. The area of infarction seen on axial CT slices is typically comma shaped, with the rostral aspect in the head of the caudate and anterior limb of the internal capsule and the tail in the putamen (fig. 1). The dimension at the point of maximal diameter is usually at least 3 cm. Lacunes may also occur in the territory of the lateral striate arteries but they are restricted to the territory of a single penetrating vessel, are therefore of smaller size (usually less than 1.5 cm diameter) and in this region usually do not produce symptoms.

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Recognition of striatocapsular infarction is important, since it differs from lacunar infarction (Donnan *et al.*, 1982; Fisher, 1982; Mohr, 1982) by virtue of involvement of large extracranial and intracranial vessels in the mechanisms of infarction. The development of infarction in the cases demonstrated angiographically depends either on complete proximal occlusion of the middle cerebral artery with adequate colateral flow to the overlying cortex via transcortical and transdural anastomoses (Vander Eecken and Adams, 1953), thus restricting the area of infarction to the deep subcortex or, alternatively, during partial embolic occlusion of the middle cerebral artery origin, the lateral striate artery origins may be blocked but allow adequate flow past the occlusion (Bladin and Berkovic, 1984).

We have now studied prospectively 50 consecutive patients with striatocapsular infarction over a 10 yr period and present here a complete account of the clinical and radiological features, neuropsychological characteristics, associated risk factors and long-term prognosis of this important stroke syndrome. By studying angiographic findings and risk factors, 4 pathophysiological subtypes have been identified.

PATIENTS AND METHODS

Patients were identified prospectively as a part of an on-going stroke subtype categorization in the Austin Hospital Stroke Unit (Chambers *et al.*, 1983). During the period of study, from July 1977 to November 1988, 3630 patients entered the Stroke Unit and were evaluated accordingly. From these, 50 patients were selected on the basis of the recent onset of an acute focal neurological deficit which related to the finding of an area of hypodensity in the striatocapsular region consistent with recent infarction. The area of infarction had to include at least two elements of the striatocapsular area: head of caudate plus internal capsule or putamen plus internal capsule. Patients with small infarcts of 1 5 cm diameter or less consistent with lacunar infarction were excluded (Bladin and Berkovic, 1984).

All patients were examined neurologically by one of us (G.A.D., P.F.B , S.F.B.) where particular care was paid to the bedside examination of cortical function. A minimum assessment included tests for neglect (visual, sensory and motor) dyspraxia (motor, ideational, dressing), speech (spontaneous, reception, expression, repetition, reading, writing), frontal perseverative tasks and graphic tests of construction. Approximately 6 months after admission a neuropsychological examination was performed on 12 consecutive patients who had exhibited cortical signs in the acute phase. The purpose of the chronic phase study was to attempt to document any residual subtle neuropsychological deficits (Skyhøj Olsen *et al.*, 1986, Vallar *et al.*, 1988) and to characterize these further. This aspect of the study may shed further light on the underlying mechanism of genesis of the acute neuropsychological changes observed (*see* Discussion for fuller review).

CT scans were performed on admission (noncontrast) and 7–10 days later (contrast enhanced). EEG was performed in the majority of patients, usually shortly after admission. In cases of identified striatocapsular infarction this was repeated 2 wks later if the first EEG was abnormal. Cerebral angiography was only performed on those patients in whom a reasonable clinical recovery had occurred in the period shortly after the stroke (from 1 to 4 wks) and in whom the pathophysiological mechanism of infarction was considered likely to relate to the extacranial vasculature by virtue of the presence of cervical bruits or other clinical indicators such as preceding transient ischaemic attacks or amaurosis fugax.

After discharge all patients were followed up in the Stroke Unit Outpatient Clinic at regular intervals. This was continued during the study period, except for 3 patients in whom telephone contact was necessary because of geographic isolation. The following categories of final outcome were used: (1) resumption of normal lifestyle and/or employment; (2) unable to resume normal activities, but managing at home (functional recovery); (3) total dependence with nursing care required (no functional recovery).

In order to determine whether patients with striatocapsular infarction had a markedly different risk factor profile than other forms of ischaemic stroke, 2 control patients for each case of striatocapsular infarction were selected who were the next sequential admissions to the Stroke Unit of the same sex and age (±5 yrs).

These had a clear diagnosis of ischaemic stroke For smoking as a risk factor, a similar comparison was made with the number of current smokers in patients with ischaemic stroke entering the Stroke Unit from 1985 to 1986. This was done because patients before 1985 were not questioned closely concerning their smoking habits, unlike the patients with striatocapsular infarction who were questioned specifically as a part of the current study.

Statistical analysis were performed using the χ^2 test and, where appropriate, applying Yates' correction for continuity (Yates, 1934). For the case control section of the study, odds ratio with 95% confidence intervals were calculated. For other nonparametric distributions, the Mann-Whitney test was used.

RESULTS

Of the 50 patients with an acute stroke due to striatocapsular infarction, there were 27 males and 23 females. This represented 1.4% of all Stroke Unit admissions during the period 1977–1988. Mean age was 63 (range 24–88) yrs.

Clinical features

The most common presentation, observed in 31 patients (62%), was that of a single event producing a hemiparesis and hemisensory loss with accompanying cortical features (Table 1). Ten patients had preceding transient ischaemic attacks in the ipsilateral carotid artery territory. Two of these had repetitive bursts of hemiplegia before the stroke within a brief period (1 had 5 events within 3 h and the other 10 events over 2 days) similar to the 'capsular warning syndrome' seen before lacunar stroke (Donnan *et al.*, 1982; Donnan and Bladin, 1987). One patient was standing at the race-course when he suddenly fell to the ground unconscious and on recovery several minutes later had right arm weakness and slurred speech. Another patient was observed to have a gradual and smooth progression of right face, arm and leg weakness to complete hemiplegia over 24 h, while 6 others had a stuttering progression over approximately the same period. The patient who developed striatocapsular infarction associated with cardiac surgery awoke from the anaesthetic with a dense right hemiplegia and aphasia.

TABLE 1. PRESENTING CLINICAL FEATURES IN 50 PATIENTS WITH STRIATOCAPSULAR INFARCTION

Presenting clinical features	*No of patients*
Single event	31
Preceding TIAs (ipsilateral)	
Hemisphere	5
Capsular warning syndrome	2
Amaurosis fugax	3
Stuttering progression	6
Gradual progression	1
Postcardiac surgery	1
Collapse and loss of consciousness	1
Total	50

The most common pattern of weakness was mainly upper limb (21 patients, 42%), then face, arm and leg equally (13 patients, 26%) followed by a variety of other patterns, including mainly facial weakness (14 patients, 28%), dysarthria only (1 patient), and arm clumsiness only (1 patient); 30 patients (60%) had sensory, as well as motor involvement, while the remaining 19 (38%) had only motor signs and 1 dysarthria only. Cortical signs, such as dysphasia, dyspraxia, motor or sensory neglect were present in 35 patients (70%). Acute ipsilateral eye deviation was seen in 4 patients.

CT characteristics

In general, the topography of the striatocapsular infarcts were similar to those that we described previously (Bladin and Berkovic, 1984) (fig. 1), although several were larger, particularly in the paraventricular region, and reached dimensions of width 3 cm, depth 5 cm and length 4.5 cm. Several scans showed incomplete involvement of the striatocapsular region, e.g. involvement predominantly of the head of caudate (figs 2A, 3A) or putamen and capsule (fig. 4A). Surrounding oedema may have contributed greatly to the hypodensity in the case shown in fig. 2A, since a later scan was quite normal despite the persistence of subtle motor signs in the right upper limb. Scans repeated in other cases showed collapse of the infarct to a slit-like structure indistinguishable from old subcortical haemorrhage (fig. 5A, B).

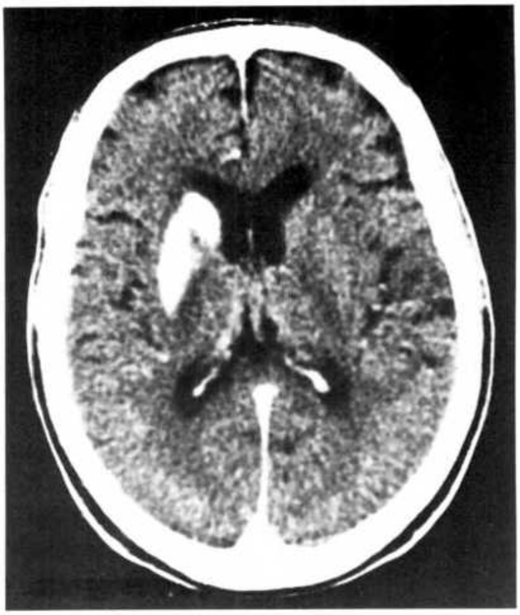

FIG. 1. Typical striatocapsular infarction showing involvement of head of caudate, anterior limb of internal capsule and putamen (contrast enhanced scan with right striatocapsular infarction in 63-yr-old male).

While the very large infarcts always produced a dense hemiplegia at onset and the few examples of incomplete striatocapsular involvement produced an incomplete syndrome (e.g., arm clumsiness only, in the case shown in fig. 2A), the topography of infarction was a poor predictor of the pattern of weakness at presentation.

Risk factors

A higher proportion of patients had cardiac disease as compared with the control patients with other forms of cerebral ischaemia (Table 2): 25 of the 50 patients with striatocapsular

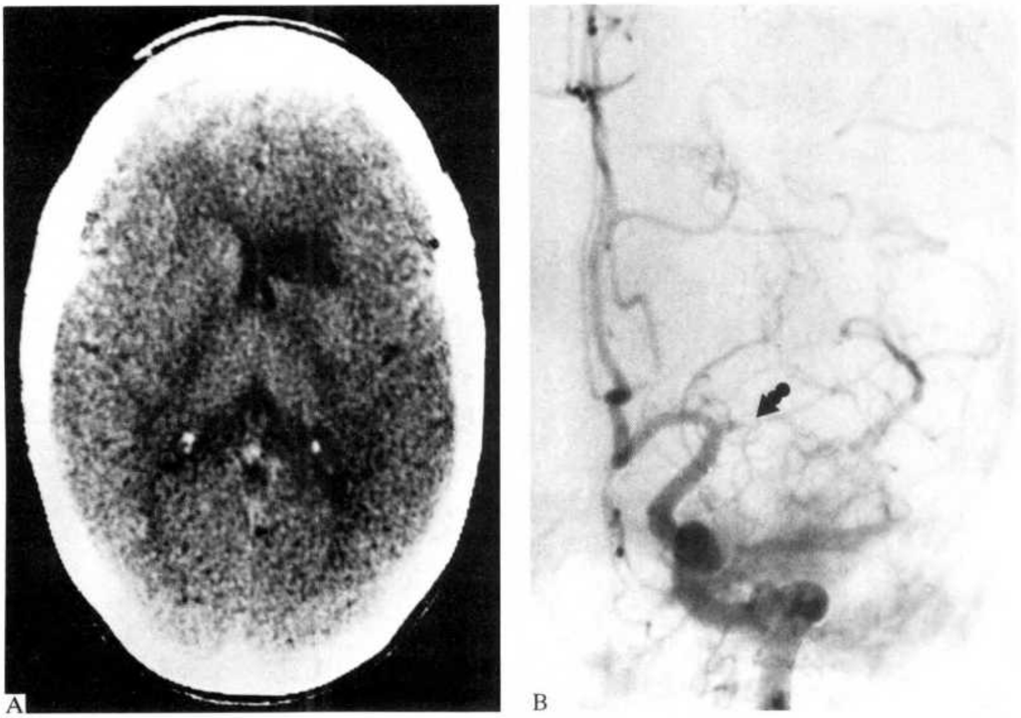

FIG. 2. A, partial left striatocapsular infarction in a 24-yr-old woman. B, angiogram showing middle cerebral arterial changes consistent with dissection or arteritis (arrow).

infarction (50%) had either atrial fibrillation (15 patients), cardiomyopathy (1 patient), ischaemic heart disease (14 patients) or a combination of these compared with 16 of 100 control patients (16%) (Odds Ratio (OR) 5.25, 95% Confidence Intervals (CI) 2.43–11.3, χ^2 19.4 for 1 df, $P < 0.001$). Five of the patients with striatocapsular infarction had had recent coronary artery bypass grafts and 46% were current smokers compared with 27% of controls (OR 2.30, CI 1.13–4.69, $\chi^2 = 5.4$ for 1 df, $P < 0.05$). The risk factors of hypertension (OR 0.70, CI 0.35–1.38), peripheral vascular disease (OR 0.85, CI 0.21–3.43), diabetes (OR 0.73, CI 0.19–2.90) and ipsilateral cervical bruit (OR 0.78, CI 0.23–2.63) did not differ between the two groups.

Cerebral angiography and relationship to cardiac risk factors

Twenty-seven patients (54%) underwent cerebral angiography (Table 3). Twelve patients (24%) had either complete internal carotid occlusion (fig. 4B) or greater than 95% stenosis ipsilaterally (fig. 6B). All 6 of the latter went on to have a carotid endarterectomy. Three patients had significant disease of the ipsilateral middle cerebral arteries in the absence of arterial change elsewhere. One had middle cerebral artery stenosis at the level of the lateral striate arteries and another had an aneurysm just beyond these arteries. The third patient was a 24-yr-old woman with arterial change in the proximal middle cerebral artery consistent with either arteritis and/or arterial dissection

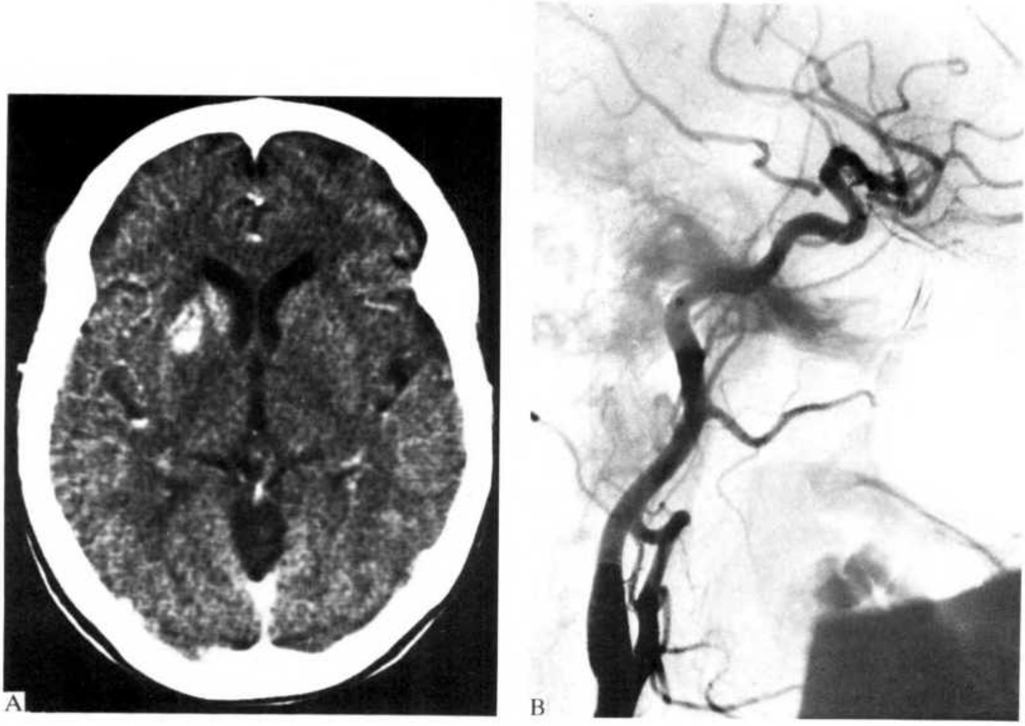

FIG. 3. Right striatocapsular infarction in a 67-yr-old male. A, contrast enhanced scan showing that the infarct mainly involved the caudate and anterior limb of the internal capsule. B, angiography was normal.

(fig. 2B). Twelve patients had normal angiograms or minimal change (fig. 3B). The timing of angiography in this group was not different from the remainder, i.e., ranging from 10 to 25 days poststroke. In the 23 patients in whom angiography was not performed, 20 had significant cardiac risk factors: 11 had atrial fibrillation, 4 had atrial fibrillation associated with ischaemic heart disease, 4 had ischaemic heart disease alone and 1 had cardiomyopathy. Of the remainder, only 6 of 27 patients had cardiac disease. In the 12 patients in whom angiography showed no change or minimal change, 4 (33%) had cardiac disease compared with only 2 of 15 in the group with severe carotid occlusive disease.

Cortical signs and EEG: relationship to angiography

Of the 50 patients 35 (70%) had cortical signs at presentation (*see* Methods). None of these had CT evidence of cortical infarction. An EEG was performed on 41 patients and focal ipsilateral slow waves were seen in 27 (66%). In general, EEG changes tended to parallel focal cortical signs in the ipsilateral hemisphere ($\chi^2 = 12.7$; $P < 0.001$) (Table 4), although it was of interest that 5 of 15 patients with no cortical signs at bedside examination had abnormal EEGs over the infarcted hemisphere, suggesting that the EEG

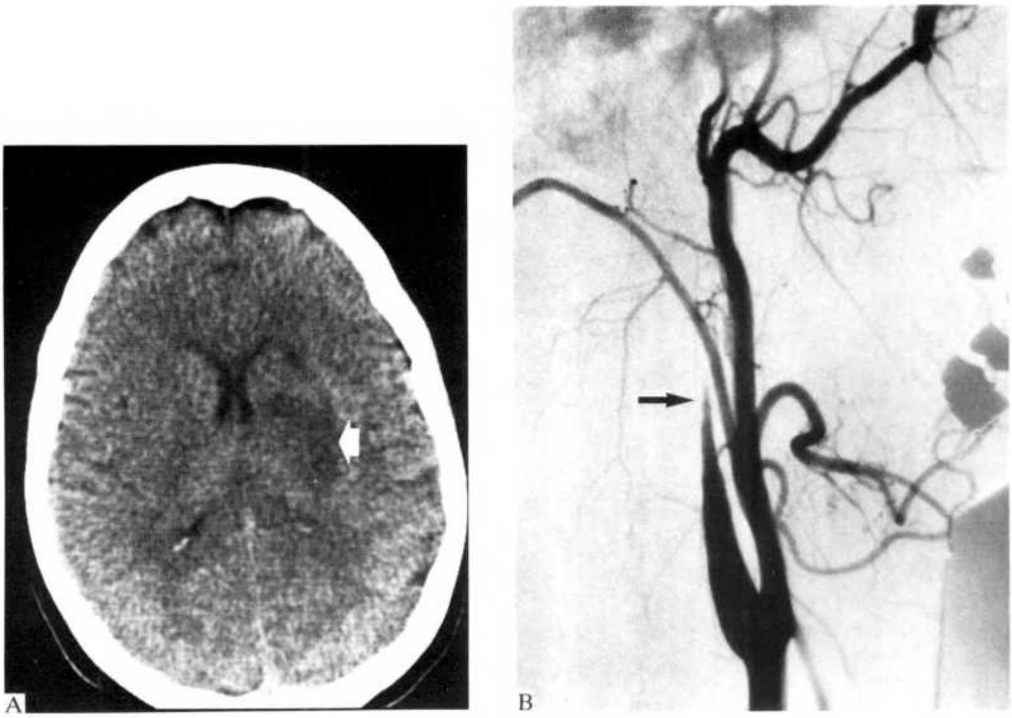

FIG. 4. A recent left striatocapsular infarction in a 35-yr-old woman. A, the infarct involved mainly the putamen (arrow) and anterior limb of the internal capsule. B, angiogram showing complete occlusion of the left internal carotid artery (arrow).

may be sometimes more sensitive than the bedside examination in detecting generalized hemisphere cortical dysfunction.

Of the 27 patients who had angiography, patients with complete internal carotid occlusion or greater than 95% stenosis were more likely to have cortical signs that those with no major extracranial carotid occlusive disease ($\chi^2 = 13.7$, $P < 0.001$) (Table 5). This was not the case with EEG changes, where focal slowing was seen as often ipsilateral to severe as to minimal carotid occlusive disease.

Neuropsychological changes

Acute. The distribution of cortical signs seen during the acute phase is shown in Table 6. Cortical signs were generally consistent with the laterality of the infarction. Examples of bedside testing included dysgraphia in a patient with left hemisphere infarction (fig. 7) and left neglect on clock construction in a patient with right hemisphere infarction (fig. 8). Frontal perseverative signs were sometimes present (fig. 9), presumably due to interruption of frontal connections in the anterior limb of the internal capsule. Of interest is the presence of right neglect without language signs in 2 of the left hemisphere patients.

Chronic. The gross language and visuospatial features seen in the acute phase had resolved. However, some degree of neuropyschological dysfunction was evident in the

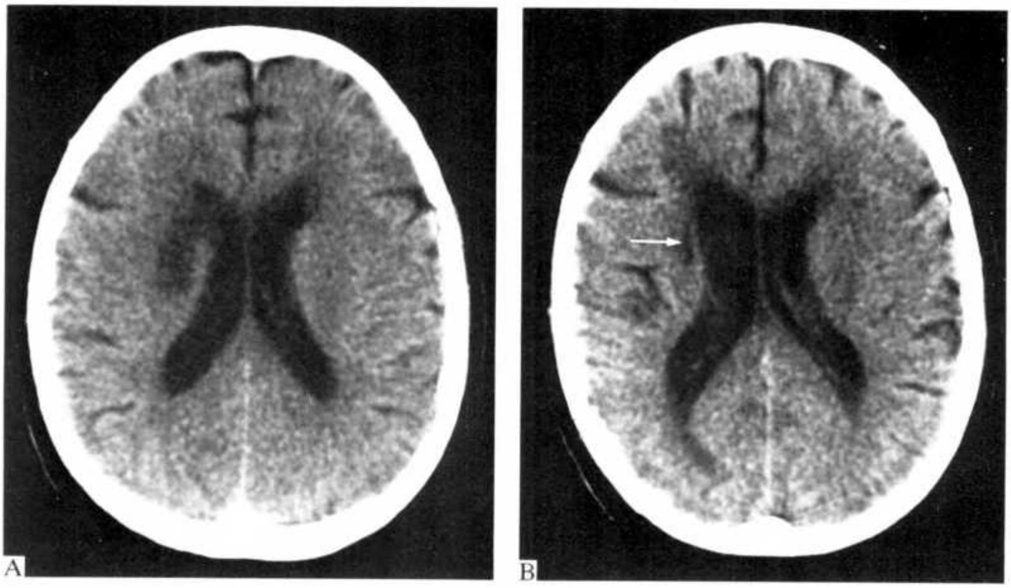

FIG. 5. A, unenhanced CT scan showing upper portion of a right striatocapsular infarct in the paraventricular region several days after the onset of left hemiparesis. B, repeat scan 3 months later showing collapse of the infarct to a slit-like structure (arrow).

TABLE 2. RISK FACTOR COMPARISON BETWEEN STRIATOCAPSULAR PATIENTS AND AGE AND SEX-MATCHED-CONTROLS WITH OTHER FORMS OF CEREBRAL ISCHAEMIA

Risk factor	*Patients with striatocapsular infarction** *(n = 50)*	*Control patients with other forms of cerebral infarction*** *(n = 100)*
Cardiac disease		
Atrial fibrillation	15	11
Ischaemic heart disease	9	5
Cardiomyopathy	1	0
Total	25 (50%)	16 (16%)[a]
Hypertension	25 (50%)	59 (59%)
Current smokers	23 (46%)	27 (27%)[b]
Diabetes	3 (6%)	8 (8%)
Peripheral vascular disease	3 (6%)	7 (7%)
Cervical bruits (ipsilateral)	4 (8%)	10 (10%)

* Mean age 64 ± 13 (SD) yrs, range 24–88 yrs ** Mean age 64 ± 13 yrs, range 20–84 yrs. [a] $P < 0.001$; [b] $P < 0.05$. Types of cerebral infarction in the control group were as follows: cortical or cortical plus subcortical 60, lacunar 25, hindbrain 11, watershed 4.

chronic phase (Table 7, Appendix). First, both patient groups tended to achieve low scores on a coding task (Digit Symbol Substitution), which in the case of the right hemisphere group differed significantly from the control group. This task is particularly sensitive to cerebral impairment, being dependent on response speed, sustained attention

TABLE 3. RELATIONSHIP BETWEEN CEREBRAL ANGIOGRAPHY AND THE PRESENCE OF CARDIAC DISEASE IN THE 50 PATIENTS WITH STRIATOCAPSULAR INFARCTION

Angiography	*n*	*No. of patients with cardiac disease*
Ipsilateral internal carotid artery		
Normal or Minimal change	12	3
Occlusion	6	1
90% + stenosis	6	1
Middle cerebral artery		
Aneurysm	1	0
Stenosis (50%)	1	0
Dissection/arteritis	1	0
Total	27	5
Not done	23	20
Total	50	25

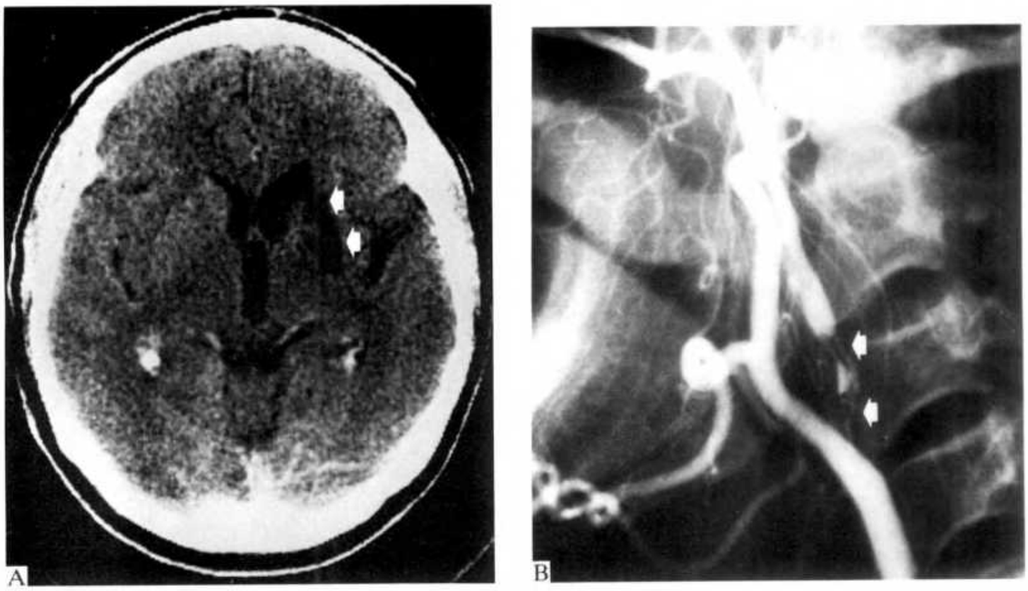

FIG. 6. A, unenhanced CT scan showing left striatocapsular infarction (arrows). B, angiogram showing ipsilateral high grade internal carotid artery stenosis (arrows).

and persistence; it is of no lateralizing significance, and is *relatively* independent of intellectual factors or learning (Lezak, 1983).

Secondly, the patients with left hemisphere infarctions had difficulty with the oral production of words starting with a designated letter, as measured by the Controlled Oral Word Association Test. This word-finding task is regarded as a sensitive indicator of 'higher level' language dysfunction in clinically nonaphasic patients, particularly if there is left or bilateral prefrontal involvement (*see* Walsh, 1987, for review). By

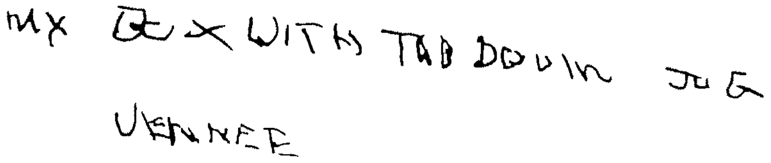

FIG 7. Dysgraphia shown by a 57-yr-old woman with left striatocapsular infarction She was right-handed and used her nondominant hand (left). The sentence was to read 'Pack my box with twelve dozen jugs of liquid veneer'

TABLE 4 RELATIONSHIP BETWEEN CORTICAL SIGNS AND EEG IN 50 PATIENTS WITH STRIATOCAPSULAR INFARCTION

	Cortical signs	*No cortical signs*
Focal EEG changes ipsilaterally	23	4
EEG normal	2	9
Bilateral EEG changes	2	1
EEG not done	8	1
Total	35	15

TABLE 5 RELATIONSHIP BETWEEN ANGIOGRAPHY PERFORMED IN 27 PATIENTS AND EEG/CORTICAL SIGNS

	Angiography	
	Carotid occlusion or high grade stenosis ipsilaterally (no. of patients)	*No haemodynamically significant change (no. of patients)*
Cortical signs clinically		
Focal hemisphere abnormalities	15	3
Normal	0	9
Total	15	12
EEG findings		
Focal ipsilateral showing	9	8
Normal	2	6
Generalized slowing	1	1
Total	12	15

TABLE 6. DISTRIBUTION OF CORTICAL SIGNS IN THE ACUTE PHASE*

Left-sided lesion	*n*	*Right-sided lesion*	*n*
Aphasia only	13	Neglect only	8
Aphasia + ideomotor apraxia	1	Neglect + anosognosia	2
Aphasia + Gerstmann's syndrome	1	Neglect + constructional apraxia	2
Aphasia + right neglect	3	Neglect + anosognosia + Constructional apraxia	2
Right neglect only	2		

* n = 34, details of the cortical findings in 1 patient were not available.

constrast, the right hemisphere patients were impaired on reasonably complex block construction task (Block Design), suggesting the presence of residual visuospatial difficulties in this group.

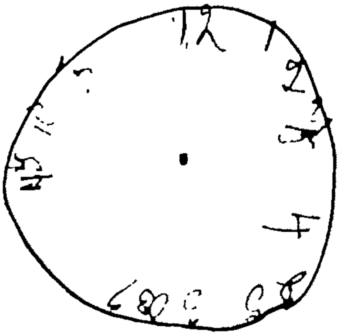

FIG. 8 Left neglect shown in clock drawing by a 65-yr-old man with right striatocapsular infarction.

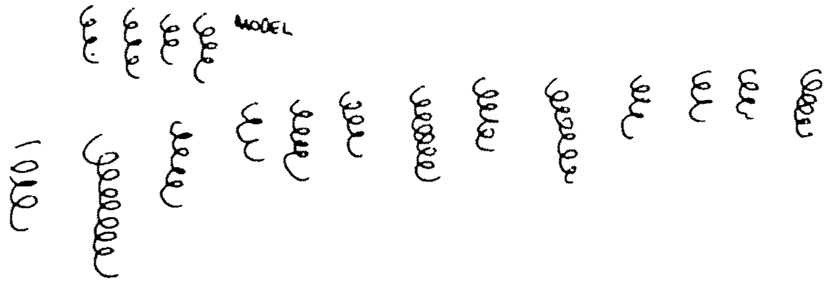

FIG. 9. Perseveration shown in the number of springs and number of coils within each spring copied from the model (*top row*). This 35-yr-old woman had a left striatocapsular infarction. (CT scan and angiography shown in fig. 4.)

Thirdly, the chronic phase was characterized by material-specific impairment in new learning in both patient groups. Patients with left hemisphere infarctions were able to learn a list of 15 words (Rey Auditory Verbal Learning Test (RAVLT)) almost as well as the control subjects or right hemisphere patients; however, their spontaneous recall of the list was markedly reduced after an interpolated interference condition (trial B of the RAVLT; *see* Table 7). They also exhibited subtle problems in recalling prose passages (Logical Memory) when compared statistically with the other two groups. Patients with right hemisphere infarctions showed impaired learning of visuospatial information, exemplified by their failure to learn the Milner Pathway of the Austin Maze; an examination of the error date in Table 7 reveals that, unlike the controls and left hemisphere patients, the patients with right hemisphere lesions maintained high error rates over 10 successive learning trials. Right hemisphere patients also differed from controls in terms of their ability to reproduce complex visuospatial information from memory (Rey's Complex Figure). This latter finding should be regarded as suggestive only, since the performance of the left hemisphere groups was approximately intermediate between that of the right hemisphere and control subjects.

Outcome

During a follow-up period of 1 month to 8 yrs (mean 2.25 yrs), 11 patients had further progression of their deficit in hospital. Two patients were lost to follow-up after being

TABLE 7 NEUROPSYCHOLOGICAL TEST PERFORMANCES OF CONTROL SUBJECTS AND PATIENTS WITH LEFT OR RIGHT-SIDED STRIATOCAPSULAR INFARCTIONS

	Controls *(n = 5)* *(mean ± SD)*	*Left infarcts* *(n = 6)* *(mean ± SD)*	*Right infarcts* *(n = 6)* *(mean ± SD)*
WAIS-R (scaled scores)			
Picture completion	9.4 (2.4)	9.3 (3.2)	9.4 (2.4)
Digit symbol substitution	9.4 (2.2)	6.8 (2.9)	7.0 (1.1)[a]
Block Design	9.4 (3.2)	10.6 (1.9)	7.3 (3.5)[c]
WMS			
Logical memory	14.6 (3.4)	11.4 (3.2)[b]	14.8 (2.5)
Rey Auditory Verbal Learning Test (Correct responses out of a total of 15)			
Trial 1	4.6 (1.7)	5.4 (2.7)	4.6 (1.5)
2	6.6 (1.7)	6.0 (3.0)	7.8 (2.2)
3	9.0 (1.0)	8.4 (3.5)	8.6 (3.1)
4	10.4 (1.1)	8.2 (3.5)	10.2 (1.8)
5	11.0 (2.6)	8.4 (3.1)	10.8 (1.8)
B	5.0 (1.4)	4.6 (1.5)	6.2 (4.6)
6	8.8 (3.5)	4.0 (2.9)[b]	10.0 (1.2)
Recognition	13.6 (1.1)	11.8 (2.6)	13.8 (1.5)
Rey's Complex Figure (Units correct out of a total of 36)			
Copy	32.2 (4.7)	33.7 (3.8)	29.8 (4.2)
Recall at 5 min	18.6 (6.7)	13.3 (2.9)	9.5 (6.5)[a]
Benton Controlled Oral Word Association Test (percentiles)	66.8 (29.8)	14.8 (26.1)[a]	43.6 (19.1)
Austin Maze (Milner Pathway) (errors)			
Trial 1	17.6 (5.0)	16.6 (5.0)	20.6 (6.2)
2	11.8 (2.2)	14.8 (11.1)	19.2 (6.2)
3	13.4 (9.5)	10.6 (4.6)	21.6 (7.8)
4	9.8 (5.3)	10.6 (9.2)	19.2 (6.6)[a]
5	9.0 (6.9)	12.2 (11.9)	17.4 (7.5)
6	7.8 (8.9)	8.4 (6.6)	16.6 (7.1)
7	8.8 (6.2)	7.0 (4.3)	16.8 (7.3)[b]
8	5.4 (4.2)	6.0 (4.3)	18.0 (7.1)[b]
9	6.8 (8.0)	6.2 (5.8)	19.0 (6.7)[b]
10	3.6 (4.0)	6.6 (6.8)	15.0 (6.9)[a]

[a] Differs from control group only (5% level, Mann-Whitney test) [b] Differs from remaining two groups (5% level, Mann-Whitney test) [c] Differs from remaining patient group (5% level Mann-Whitney test)

transferred back to their countries of origin. One patient had a brief TIA affecting the same side of the body as the previous stroke 2 yrs earlier. Another patient, who was in chronic atrial fibrillation, developed a striatocapsular infarct on the opposite side 5 yrs later. Eight patients died, 5 from cardiac conditions, 1 from renal failure and 2 from uncertain causes. Stroke or vascular death rate for the follow-up period was therefore approximately 2.7% per yr.

Overall, the majority of patients either had functional recovery (23 patients) or a more complete recovery with return to work or normal lifestyle (15 patients). Twelve patients had no functional recovery. Although numbers were low for an angiographic correlation with outcome, a suggestion that minimal change or normal angiography was associated with a better outcome was seen, since 13 of 15 such patients had a functional recovery or return to work. Four of the 6 patients with carotid occlusion made a functional recovery, but none was able to return to work or its domestic equivalent.

Patients who were able to return to work to resume normal lifestyle were significantly younger (56.9 ± yrs) compared with those with no functional recovery (69.9 ± yrs) (two-tailed Mann-Whitney test, U = 37, $P < 0.02$) and fewer had cortical signs at presentation (8/15 vs 2/12, $\chi^2 = 3.8$, $P < 0.05$). Topography of infarction at the onset of stroke was not a good predictor of outcome excepting for the few cases with only partial striatocapsular infarction (all returned to work) or massive infarction (3 cases, all with no functional recovery). The pattern of motor weakness at onset did influence outcome, since 10 of the 12 with no functional recovery had hemiplegia equally affecting face, arm and leg while this pattern was present in only 3 of the 15 who returned to normal lifestyle ($\chi^2 = 8.3$, $P < 0.01$). Brachiofacial weakness or mainly arm weakness was the commonest pattern in the latter group. The presence of cardiac or other risk factors had no impact on outcome.

DISCUSSION

The current study expands on our previous findings concerning the clinical features and presentation of patients with striatocapsular infarction (Bladin and Berkovic, 1984). The most common presentation is that of a single episode of the sudden onset of a hemiparesis affecting the upper limb more than lower limb or face, with accompanying sensory and cortical signs. The neuropsychological features frequently include language disturbances or neglect attributable to the affected hemisphere, together with difficulties with new learning tasks, which may persist for a greater period than has previously been appreciated. Less frequently a pure motor hemiparesis with minimal cortical signs may be seen and rarely, subtle change such as dysarthria alone or merely upper limb clumsiness. An examination of risk factors associated with striatocapsular infarction in this study shows that this form of stroke is associated with cardiac disease more than all other forms of ischaemic stroke combined. Fifty per cent of patients had either atrial fibrillation, cardiomyopathy, ischaemic heart disease or a combination of these, compared with 24% of controls. Five patients had had recent coronary artery bypass grafts. Smoking was the only other significant risk factor greater than other forms of ischaemic stroke, perhaps because of its strong association with cardiac disease (Doll and Peto, 1976).

By taking into account the cardiac risk factors described and the angiographic findings, 43 cases of striatocapsular infarction studied could be divided into four pathophysiological subgroups. Seven patients had less adequate investigation and could therefore not be classified.

Groups 1. Presumed cardiac source of embolus (17 patients)

All patients in this group had relatively severe cardiac disease which may have acted as a potent source of embolism. This was recognized by Santamaria *et al.* (1983) who reported 8 patients with deep subcortical infarction secondary to cardiac embolism and in our population was certainly the most common mechanism of development of striatocapsular infarction. Fifteen of our patients had atrial fibrillation with or without ischaemic heart disease and 1 had cardiomyopathy. All patients had a single abrupt clinical event without premonitory symptoms. The embolus most likely lodged, either transiently or permanently, at the origin of the middle cerebral artery, as demonstrated in one of these cases reported previously (Bladin and Berkovic, 1984). On these occasions, collateral flow from the anterior cerebral artery may take over much of the function of the superior division of the ipsilateral middle cerebral artery while collateral flow from the posterior circulation may supply the territory of the inferior division of the middle cerebral artery and thus preserve cortical tissue (Vander Eeken and Adams, 1953; Bladin and Berkovic, 1984). If the arterial occlusion is only temporary, cortical flow may also be restored via anterograde flow through the middle cerebral artery stem.

Group 2. Severe carotid occlusive disease (12 patients)

A haemodynamically significant ipsilateral high grade internal carotid artery stenosis was seen in 6 patients and complete occlusion in a further 6 patients. One of the former had a long intraluminal clot, the details of which have been described previously (Donnan and Bladin, 1979). Five patients had premonitory events (3 with amaurosis fugax) and 2 had a stuttering onset, confirming previous observations that severe carotid occlusive disease may be associated with premonitory and intermittently progressive symptoms (Fisher, 1951), unlike cerebral infarction due to cardiac emboli where this pattern is unusual (Dyken *et al.*, 1986). Cardiac disease was present in only 2 of these patients, confirming the paramount role of the extracranial vascular disease in the pathogenesis of striatocapsular infarction in this group. The mechanism of infarction may involve emboli from the stenotic, occluded or occluding internal carotid artery which may lodge at the middle cerebral artery origin as in the first group. However, in the angiograms studied, crossflow from the opposite hemisphere via the anterior communicating artery to the ipsilateral middle cerebral artery was commonly the means by which hemispheric flow was restored as well as via anastomotic flow from anterior and posterior cerebral arteries. Whether deep striatocapsular infarction can occur in these cases based entirely on reduced middle cerebral artery flow and its effect on the lenticulostriate end arteries without involving embolic occlusion as a mechanism is uncertain.

Group 3. Normal angiography (or minimal change), mechanism uncertain (11 patients)

This group comprises those patients in whom angiography was normal or only minimal change was present. Three patients had ischaemic cardiac disease and it is conceivable that cardiac embolism was responsible for the striatocapsular infarction in these cases. Indeed this mechanism must remain a possibility in other cases with relatively normal angiograms, since the cardiac disease or dysrhythmia may remain occult. However, 5 patients had premonitory symptoms and 2 had a stuttering progression of deficit, perhaps making a cardiac source of embolism less likely. Two patients presented with the bursts of hemiplegia characteristic of the 'capsular warning syndrome' previously described as a prelude to lacunar infarction (Donnan *et al.*, 1982; Donnan and Bladin, 1987), but on these occasions evolved into striatocapsular infarction involving multiple rather than single lenticulostriate vessels. In 2 patients there were features in the clinical presentation which suggested that vasospasm may have played a role; emotional stress was present before the event, mild throbbing headache was noted during and after the event and a 'washed out' sensation persisted for many hours later. Another patient lost consciousness for a brief period at the onset, suggesting a more global circulatory disturbance during the infarct genesis.

Groups 4. Middle cerebral artery abnormalities (3 patients)

Here disease of the middle cerebral artery was seen in the absence of cardiac or extracranial occlusive disease. In one 24-yr-old woman, middle cerebral artery dissection or arteritis was suspected with resultant occlusion of the origins of several penetrators to the striatocapsular region (fig. 2). Similar occlusion of the origins of penetrators was likely in the patient where atheromatous middle cerebral artery stenosis of approximately 50% was seen. The single patient in whom an aneurysm was demonstrated distal to the lateral striate arteries did not have evidence of subarachnoid haemorrhage, so the relationship of the aneurysm to striatocapsular infarction must remain speculative. While middle cerebral artery disease was a less common mechanism of striatocapsular infarction in our series, this may not be the case in Japanese and American black populations where middle cerebral artery occlusive disease is said to be more common (Kieffer *et al.*, 1967; Gorelick *et al.*, 1984).

There is obvious confusion in the literature concerning the nature and terminology of subcortical infarctions. We suggest that the term striatocapsular infarction be used for the clearly recognizable infarctions restricted to the territory of the lenticulostriate vessels of the middle cerebral artery with its typical clinical correlates as described here. A clear distinction may then be made from other 'deep subcortical' infarction such as in the anterior choroidal artery territory (Decroix *et al.*, 1986), lacunar (Fisher, 1982; Mohr, 1982) and internal watershed infarctions (Wodarz, 1980; Bogousslavsky and Regli, 1986), all of which have differing clinical presentations, CT topography and pathogenesis.

Early studies of the pathological correlates of carotid and middle cerebral artery occlusions contained descriptions of what could now be classified as striatocapsular infarctions (Foix and Lévy, 1927; Torvik and Jörgensen, 1966; Blackwood *et al.*, 1969). The proposed mechanisms of infarction were similar to our Group 2 patients, particularly in the study of Trovik and Jörgensen (1966). Similarly, recent descriptions of patients with aphasia attributed to deep subcortical infarction contained some cases of striatocapsular infarction (Damasio *et al.*, 1982; Naeser *et al.*, 1982), although the pathogenesis of infarction was not discussed, thereby perhaps minimizing the cortical contribution to the aphasia due to depressed cortical blood flow (*see below*). Rascol *et al.* (1982) described a variety of deep subcortical infarctions of which only type I was clearly striatocapsular. In broader clinical studies of occlusion of internal carotid and middle cerebral arteries both Ringelstein *et al.* (1983) and Caplan *et al.* (1985) had some examples of striatocapsular infarction but did not focus on the clinical features of this group particularly. However, the patients described by Adams *et al.* (1983); Santamaria *et al.* (1983) and Levine *et al.* (1988) more clearly had striatocapsular infarction and had clinical features similar to our own cases.

It was of interest that ipsilateral EEG slow waves tended to parallel the presence of focal cortical signs (Table 4). Cortical signs are extremely useful in the initial bedside distinction of the two commonest forms of ischaemic hemisphere stroke, subcortical lacunes and cortical infarcts. The former rarely have cortical signs and typically have a normal EEG, whereas the latter usually have both cortical signs and focal slow waves

(Chambers *et al.*, 1983; Macdonell *et al.*, 1988). Striatocapsular infarction presents an interesting paradox; cortical signs and focal EEG slow waves are usual, and yet the anatomical lesion is exclusively subcortical. There is debate in the literature as to whether 'cortical' features in such subcortical lesion are due to dysfunction of the overlying cortex or represent true subcortical signs (Damasio *et al.*, 1982; Naeser *et al.*, 1982; Perani *et al.*, 1987; Vallar *et al.*, 1988).

Our clinical, EEG and angiographic data, together with blood flow studies of other workers, strongly suggest that the cortical signs in the acute phase are due to disruption of cortical function, rather than a subcortical effect per se. It is well known that focal slow waves in acute cortical lesions are due to involvement of the immediate subcortical white matter, resulting in cortical deafferentation; such slow waves are absent or inconstant features of lesions limited to the internal capsule or deep grey matter (Ball *et al.*, 1977; Gloor *et al.*, 1977; Macdonell *et al.*, 1988). The association of focal slow waves with cortical signs in our patients thus strongly suggests that there is functional disruption lateral to the anatomical margins of the striatocapsular lesion, involving the immediate subcortical white matter and the cortex itself. This electroclinical deduction is supported by cerebral blood flow studies showing acute focal depression of cortical blood flow in patients with striatocapsular infarction (Perani *et al.*, 1987; Vallar *et al.*, 1988).

This depression of blood flow and presumably metabolism represents, in part, the 'ischaemic penumbra' of the infarction (Astrup *et al.*, 1981). Our finding that cortical signs are more likely to be present in patients with severe extracranial carotid occlusive disease, where the ability to limit the size of the ischaemic penumbra may be reduced compared with those patients with uninterrupted anterograde flow through the internal carotid and middle cerebral systems, further supports this contention. Any role played by diaschisis due to involvement of fibres of passage to the cortex in the genesis of cortical signs (Perani *et al.*, 1987) is therefore likely to be overwhelmed by local haemodynamic factors, at least in the acute phase.

However, in the chronic phase, our neuropsychological findings would suggest that the remote effect of depressed cerebral blood flow and metabolism that follows focal ischaemia, that is, diaschisis (Høedt-Rasmussen and Skinhøj, 1964) may be responsible for the subtle, significant and persisting deficits. Amongst these deficits were material specific learning impairments which are classically due to unilateral diencephalic or medial temporal lobe lesions; both areas are supplied by the anterior choroidal and posterior cerebral arteries, well outside the territory of the vessel supplying the striatocapsular regions, the middle cerebral artery. A possible explanation for persisting deficits of this type is diaschisis involving fibre connections crossing the vascular territories of middle cerebral and posterior cerebral arteries.

Our finding that the neuropyschological impairment in the acute phase was always attributable to the affected hemisphere has been shown by others (Damasio *et al.*, 1982; Skyhøj Olsen *et al.*, 1986; Perani *et al.*, 1987; Levine *et al.*, 1988; Vallar *et al.*, 1988; Højer-Pedersen and Petersen, 1989), although Cambier *et al.* (1984) described a case of a left subcortical lesion in which autotopagnosia, amongst other neuropsychological changes, was present. However, the pathology was haemorrhage, rather than infarction, and pressure effects may have come into play.

Up until this time there has been disagreement in the literature as to whether the neuropsychological deficits associated with striatocapsular infarction may persist over a prolonged period. Levine *et al.* (1988) found that more than 75% of surviving aphasics in their series had obvious speech deficits some months after the stroke. On the other hand, Skyhøj Olsen *et al.* (1986) demonstrate that in 6 out of 8 aphasic patients clinical remission was complete at 3 months after the stroke. At 6 months, 3 of their patients showed subtle language signs on detailed neuropsychological examination. It is possible that the learning functions assessed in our study represent a more sensitive index of neuropsychological impairment, and our findings suggest that the chronic phase is not devoid of such impairment in a significant proportion of patients with purely subcortical lesions. This may be of particular importance when assessing such patients for re-entry into the workforce.

We found the outcome of patients with striatocapsular infarction appears to be intermediate between the excellent prognosis of lacunar infarction (Gandolfo *et al.*, 1986; Bamford *et al.*, 1987) and the poor prognosis of patients with cortical/subcortical infarction. The latter are faced not only with motor impediments, but also gross neuropsychological dysfunction. Two-thirds of our patients had at least a functional recovery and half of these were able to return to work. Predictors of a better outcome were of younger age, absence of cortical signs at presentation and no haemodynamically significant disease seen on cerebral angiography. A poor prognosis was likely in older patients with equal motor involvement of face, arm and leg at onset, the presence of cortical signs and, if performed, angiographic findings of severe carotid occlusive disease. Based on these findings a relatively early prediction of likely outcome should be possible in many cases.

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(Received May 17, 1989. Revised December 28, 1989. Accepted January 9, 1990)

APPENDIX

Explanatory notes for neuropsychological tests used

Picture Completion

The subject is presented with a series of incomplete pictures from which an important part is missing; the task required the missing part to be identified. As a subtest of the Wechsler Adult Intelligence Scale-Revised (WAIS-R) this test is scored in terms of the number of correct missing parts identified, and this can be converted into an age-scaled score as above

Digit Symbol Substitution

This coding task is a subtest of the WAIS-R The subject is presented with a coding table, the top row of which displays the numerals 1 to 9, while bottom row displays 9 different symbols, each of which is arbitrarily but uniquely associated with 1 of the numerals. The subject is also presented with a series of numerals ranging between 1 and 9 in a recurring random sequence He/she is required to write the associated symbol below each numeral, and is allowed to refer to the coding table throughout the task. The score is the number of symbols correctly coded in 90 s; this score can be converted to an age-scaled score with a mean of 10 and an SD of 3.

Block Design

This test is a block construction task. Each block has 2 red sides, 2 white sides and 2 red and white sides. The subject is required to replicate a series of designs. The test is timed and the score can be converted to an age-scaled score as above since it is also a subtest of WAIS-R.

Logical Memory (Wechsler, 1945)

A passage of prose is read to the subject, who is then required to recall as much of the passage as possible. The response is scored in terms of the number of ideas correctly recalled This score can then be age-scaled as for WAIS-R subtests The test is then repeated using a second passage.

Rey Auditory Verbal Learning Test (Lezak, 1983)

A list consisting of 15 common nouns is presented 5 times to the subject, and spontaneous recall is examined after each presentation. Following the fifth trial, the subject listens to a second list of 15 common nouns, none of which was contained in the previous list (trial B) The subject is then examined for spontaneous recall of the original word list (trial 6) A comparison between the fifth trials and trial 6 reflects the extent to which the interpolated list interferes with retention of the original list The test is scored in terms of the number of words correctly recalled on each trial.

Rey Complex Figure (Osterreith, 1945; Lezak, 1983)

The subject is required to copy a complex but meaningless design, and is then asked to reproduce it from memory 5 min later. In this study, the Controlled Oral Word Association Test was administered during the 5 min gap The test is scored in terms of the number of elements correctly copied or reproduced.

Controlled Oral Word Association Test (Benton et al., 1983)

This test elicits the oral production of words beginning with a designated letter. The letters used in this study were 'F', 'A' and 'S', and the subject is allowed 1 min for each letter Certain restrictions are placed on the subject, i e , proper nouns are disallowed, as are words with a common stem but different suffixes and repetitions of a word already given (*see* Walsh, 1985). The score is the total number of words allowed; the norms are standardized in terms of age and years of education, yielding percentiles.

Austin Maze (Walsh, 1985)

The subject is seated in front of 10×10 matrix of electrically activated 'stepping stone' switches, and is required to learn a predetermined rectangular pathway through the matrix. Switches which are on the pathway momentarily activate a green light and those not on the pathway activate a red light and buzzer. Milner's (1965) pathway was used in the present study and the task was scored in terms of the number of errors on each of 10 trials.

Brain (1991), **114**, 71–84

HUMAN OLFACTORY DISCRIMINATION AFTER UNILATERAL FRONTAL OR TEMPORAL LOBECTOMY

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SUMMARY

Olfactory discrimination and detection was studied in 106 patients with unilateral cerebral excision in the right or left temporal lobe, right or left frontal lobe, left parietal lobe, or right frontal and temporal lobes, and in 20 normal control subjects. Detection thresholds for n-butyl alcohol, measured separately in each nostril, did not differ across subject groups or across nostrils, thus excluding any primary sensory loss. The discrimination task involved monorhinal presentation of pairs of unfamiliar odorants, which the subjects judged as same or different in quality. The results showed a significant deficit in discrimination confined to the nostril ipsilateral to the lesion in patients with temporal lobe removals Patients with frontal lobe excisions were also impaired and, for patients with right frontal lesions including the orbital cortex, the impairment was found in both nostrils. Patients with left parietal lesions did not demonstrate any significant deficits. Normal subjects showed consistently better performance in the right than in the left nostril. The results are interpreted as reflecting the importance of the orbitofrontal cortex in olfactory discrimination Temporal lobe lesions may disrupt the input to the orbitofrontal cortex, thereby producing poorer performance. The nostril difference in the normal subjects, together with the birhinal impairment in patients with right orbitofrontal damage, suggest a relative advantage of the right orbital region in olfactory processing.

INTRODUCTION

Considerable anatomical and physiological data indicate that processing of olfactory information takes place in the anterior inferior temporal lobe and in the orbitofrontal region. It has been shown that in vertebrates the olfactory tract projects ipsilaterally from the olfactory bulb to the prepyriform cortex, together with the uncus, amygdala and entorhinal area (for review, *see* Eslinger *et al.*, 1982). According to Potter and Nauta's (1979) work on the monkey, there is an important corticocortical projection between the prorhinal region in the temporal lobe and the orbitofrontal cortex. This latter region, together with much of the rest of the orbital surface of the frontal lobe, also receives an indirect olfactory input via the dorsal medial nucleus of the thalamus (von Bonin and Green, 1949). Motokizawa and Ino (1983) also reported a direct projection in the cat between pyriform cortex and the orbital gyrus.

The importance of the orbitofrontal region to olfactory function has been suggested

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by a number of authors. Tanabe *et al.* (1975*b*) recorded from single neurons in an area they identified as lateral posterior orbitofrontal cortex (LPOF) in unanaesthetized monkeys. They reported that each unit responded only to a small number of specific odorants, and in 50% of cases just to one stimulus, whereas units in the olfactory bulb or in the amygdala responded much more generally to many odorants. These results suggest that relatively complex neural processing of odours is carried out in this region, and lead to the hypothesis that the LPOF may be crucial to discrimination of olfactory stimuli. This hypothesis was supported by another study by Tanabe *et al.* (1975*a*) showing that bilateral lesions to this region in monkeys lead to definite impairments in an odour discrimination task, whereas lesions in adjacent areas do not disturb this ability. Allen (1941) had earlier reported similar deficits in the dog following ablation of the ventrolateral frontal lobe. Furthermore, Eichenbaum *et al.* (1980) have also found olfactory discrimination deficits in rats following lesions of the orbitofrontal cortex.

The human neuropsychological literature contains relatively few studies on olfactory function. Deficits in several different types of olfactory tasks, including recognition, identification, and discrimination, have been demonstrated among patients with temporal lobe excisions (Rausch *et al.*, 1977; Abraham and Mathai, 1983; Eskenazi *et al.*, 1983, 1986; Jones-Gotman and Zatorre, 1988*a*, *b*). Most of these studies agree that such patients do not show consistent alterations in their perceptual thresholds to odorants, thereby suggesting that the deficits are not attributable to a basic sensory loss. In some studies there has been evidence for an asymmetry of function favouring the right hemisphere. For example, Abraham and Mathai (1983) found a deficit after right temporal lobectomy but not after left on a complex odour-matching task, and Rausch *et al.* (1977) reported a greater deficit following right temporal lobectomy on a delayed matching task.

Only two previous studies have examined the effects of orbitofrontal cortical lesions on olfaction. Potter and Butters (1980) studied 5 patients with a tumour or haematoma in the orbitofrontal region and 4 patients with posterior lesions. They found that the patients wtih frontal lesions were severely impaired in an odour-quality discrimination task in the nostril ipsilateral to the lesion; the 4 patients with posterior lesions performed normally. Detection ability for the frontal lobe group was reported to be slightly better than normal at one concentration of the target odorant, and comparable to normal at a higher concentration. This study provides important evidence with respect to the function of the orbitofrontal cortex, but its conclusions are limited due to the small number of subjects tested and to the lack of comparison between left and right-sided lesions.

In the only other published study of the frontal lobe contribution to olfaction (Jones-Gotman and Zatorre, 1988*a*), we examined olfactory identification in patients with frontal lobe lesions invading or sparing the orbital surface. We found that among those with orbitofrontal damage there was a significant impairment of identification ability, whereas those whose frontal lobe excision spared the orbital surface did not differ from normal. Lesions to either left or right frontal regions resulted in equivalent deficits in that study, in which the odorants were presented to both nostrils. We did not observe any alterations in detection thresholds for n-butyl alcohol, measured monorhinally, in either nostril of these subjects.

The animal data mentioned above suggest a specific role for the orbitofrontal cortex in higher-order olfactory processing, particularly discrimination. The possible role of this region in discrimination of different substances has not been adequately explored in humans, however. The possible predominance of one hemisphere over the other in such tasks has also not been examined in detail. We undertook the present study to investigate these issues in groups of patients with well-defined focal excisions.

METHODS

Subjects

The experimental subjects were 106 patients who had undergone focal unilateral cerebral excision for the relief of intractable epilepsy, and 20 normal control subjects. In all cases the preoperative cerebral lesion had been well-lateralized and, except where noted below, all lesions were static and atrophic. All patients were right-handed except for 7 who were left-handed or ambidextrous; those 7 patients had been shown by preoperative intracarotid sodium amytal tests (Branch *et al.*, 1964) to have speech represented in the left hemisphere. Patients were excluded from study if they presented atypical speech representation, had known damage outside the region of surgical excision, had a fast-growing tumour, or had a Full Scale WAIS-R IQ under 75. Fifty-three of the patients were tested 13–18 days after surgery, and the rest were seen in follow-up study one or more years after operation.

The patients were grouped originally according to side and lobe of cerebral excision, resulting in 6 patient groups: a left temporal lobe group (LT; n = 31), a right temporal lobe group (RT; n = 31), a left frontal lobe group (LF; n = 9), a right frontal lobe group (RF; n = 17), a group in whom the removal included tissue from both the right temporal and right frontal lobes (RFT; n = 10), and one group with left sided surgical lesions in the parietal or central regions (LP/C; n = 8), all outside known or suspected olfactory areas.

In all cases of temporal lobectomy the excision included the lateral neocortex together with the amygdala and uncus, and varying amounts of the hippocampal region. Of the patients with LT lesion, 12 had undergone extensive resection of the medial structures (more than 1.5 cm of the hippocampus and hippocampal gyrus), and the rest had undergone a more limited medial excision. Within the LT group there were 3 cases of low-grade glioma, and 1 of angioma. Of the patients with RT excision, 1 had a low-grade glioma, and 1 a hamartoma; 13 had undergone extensive medial resection.

The surgical removals for the patients in the LF group are shown in fig. 1. Among these patients a further classification, based on drawings made by the neurosurgeon at the time of excision, was made according to whether the damage extended into the orbital surface or spared this region Of the 9 patients with LF lesion, 6 had encroachment onto the orbital surface (LF-Orb, shown on the right of the figure) and the rest had sparing of this area (LF-Sp, shown on the left of the figure). Two patients in the LF group had an arteriovenous malformation (Cases 3, 8), and there were 3 with cerebral tumours· 1 ganglioglioma (Case 9) and 2 astrocytomas (Cases 2, 7).

Fig. 2 shows the cortical excisions in the RF group. The orbital surface of the frontal lobe was spared in 10 patients and excised wholly or partially in the remaining 8. Those in whom it was spared (RF-Sp) are shown on the left of the figure (Cases 10–18) and those with orbitofrontal encroachment (RF-Orb) are shown on the right (Cases 19–26). Included in the group are 2 cases of arteriovenous malformation (11, 17), 4 with an astrocytoma (10, 15, 16, 21), 1 oligodendroglioma (19), 1 mixed glioma (12) and 1 cystic tumour (18)

The cortical excisions in the 10 patients of the right frontotemporal group (RFT) included tissue from both the right temporal and right frontal lobes, as depicted in fig. 3. The size of the temporal lobe excisions ranged from 3.5 to 9 cm, but in all except 2 patients (Cases 31, 36) the hippocampal removal was small. The orbitofrontal cortex was spared in only 2 patients (27, 28); that area was partially or wholly removed in all of the remaining patients. There were 2 cases of cerebral tumour in this group: 1 low-grade glioma (33), and 1 astrocytoma (36).

The LP/C group contained patients with more variable left-sided surgical lesions in the parietal, posterior or central areas (fig. 4). Four of these had an arteriovenous malformation (Cases 37, 41, 43, 44), 1 had an astrocytoma (42) and 1 a low-grade glioma (39)

The 20 right-handed normal control subjects were mainly hospital support staff, students, and relatives of patients. They were chosen to match the patient groups as closely as possible with respect to age, sex,

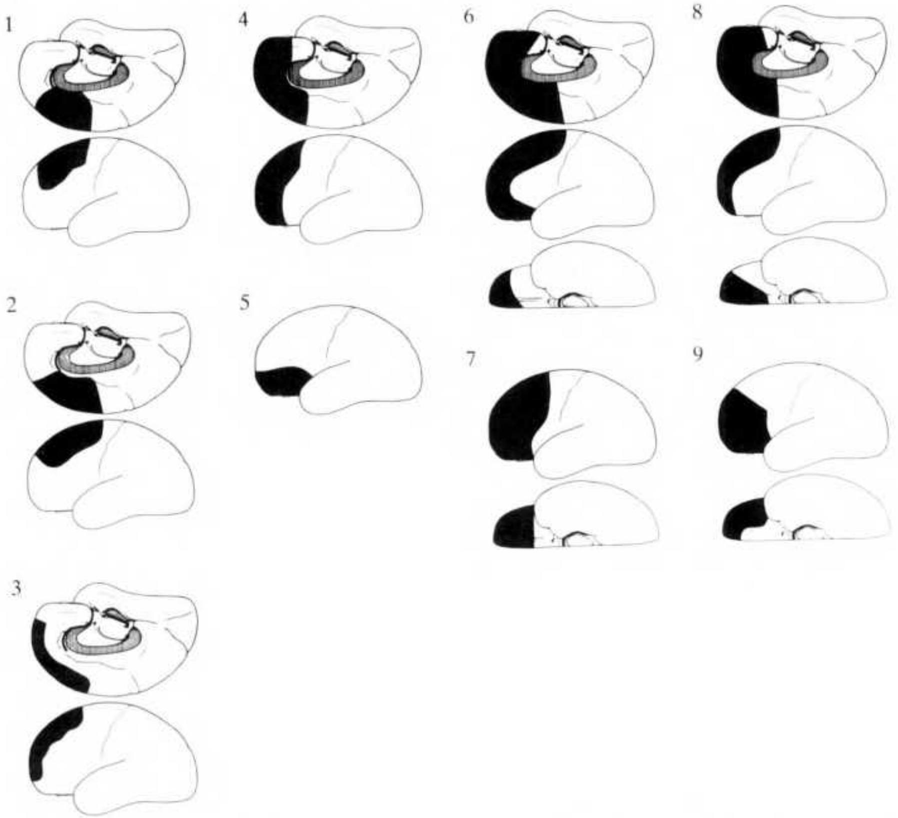

FIG. 1. Left frontal lobe group (LF). Brain maps based on the surgeon's drawings at the time of operation, showing (in black) the estimated extent of removal. Where appropriate, three views of the brain are shown, with the medial aspect above, the lateral aspect in the middle, and the ventral surface below (e.g., Case 8). For some cases, only two views were appropriate or available, and for other cases the lateral view alone is shown. The orbital surface of the frontal lobe was spared in Cases 1–3, whereas this region was encroached upon in the remaining cases (4–9).

education and socioeconomic status. Analyses of variance did not demonstrate any significant differences in age, IQ ratings, or educational level among the various subject groups.

Table 1 shows the mean age, years of education, sex distribution and Full Scale Wechsler IQ ratings for the 106 patients subdivided according to side and site of lesion, together with the same data for the 20 normal control subjects, except that no intelligence ratings were obtained for those subjects. All subjects gave informed consent for testing.

Test materials

Eight pairs of odorants were used, selected from among stimulus pairs that had been rated previously for similarity by normal subjects (Mair *et al.*, 1980). The extremes were selected for this experiment, so that the 4 pairs that had been rated most highly similar and the 4 most dissimilar were used. These are listed in Table 2.

The odorants were presented via a modified 'sniff strip' (Engen, 1965), which is a small glass rod with a wad of odour-saturated cotton wrapped around the end. For presentation, the odours were placed just under the subject's nostril, providing a more constant vapour than open bottles. The odorants were kept inside a fume adsorber, which filters air through activated charcoal, thereby preventing diffusion of odours into the testing room except during presentation.

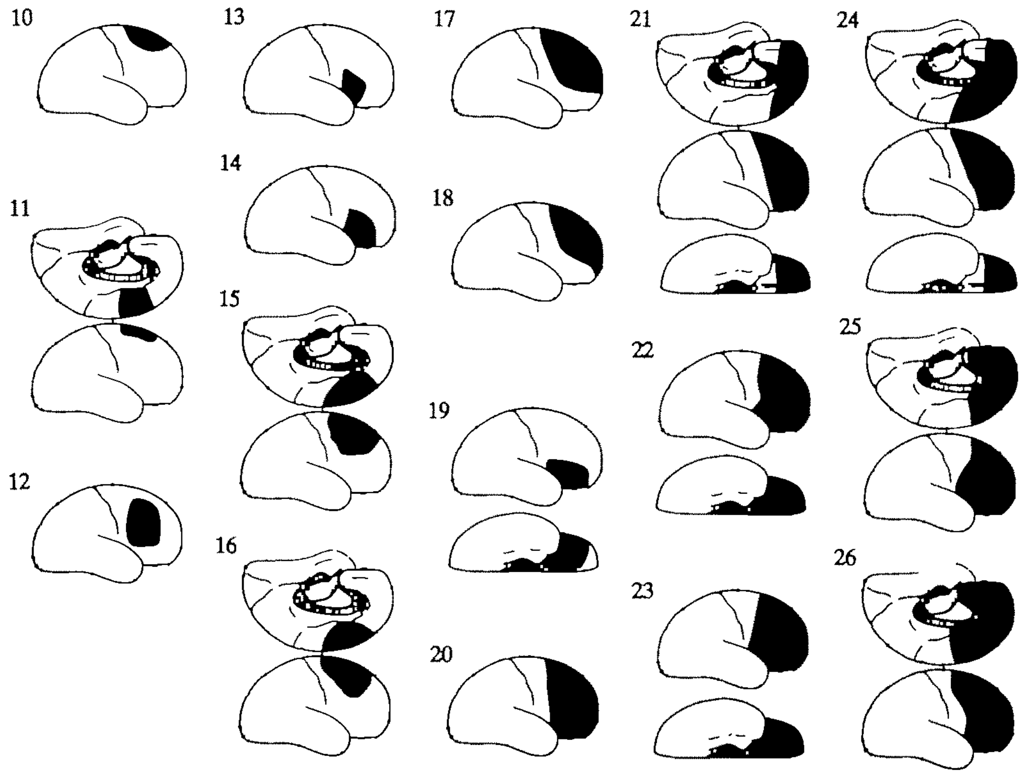

Fig 2. Right frontal lobe group (RF). Brain maps indicating estimated extent of removal (*see* fig. 1 for explanation of the different views of the brain illustrated in this figure) The excisions in Cases 10–18 spared the orbital surface of the frontal lobe, whereas in Cases 19–26 the excision encroached on this area

Procedure

Subjects received 32 monorhinal discrimination trials in a same-different paradigm. The 32 trials comprised presentation of each of the 8 odour pairs once to each nostril (16 'different' trials), and presentation of each odour of the pair with itself (16 'same' trials). The order of presentation of items within each pair was counterbalanced across nostrils; for example, if pair A-B was presented to the left nostril, pair B-A would be presented to the right nostril on a later trial. One nostril was tested on each trial by requiring the subject to hold the other nostril closed with his finger and to inhale only through the open nostril. The order of nostrils tested was randomly assigned across trials. All subjects received the same random order of trials.

Subjects were told that they would be presented with two substances on each trial, and they were to determine whether they had smelled exactly the same smell twice or if the second one was different from the first. It was emphasized that an odour quality judgment was required, as opposed to a judgment based on strength. The two odorants in each trial were presented in quick succession with a few seconds intervening between the items in a pair, and a minimum of 20 s was allowed between trials. Subjects were allowed to sniff only once for each presentation, and were then required to respond either same or different, and to guess if unsure. No feedback was given as to the correctness of the response.

A discrimination score was calculated separately for each nostril by subtracting errors ('different' responses when the items were the same) from correct answers ('different' responses when the items were different), yielding a value with a maximum of 8, and with 0 being chance performance.

Testing was carried out in the context of two other olfactory tests given in the same session, but these

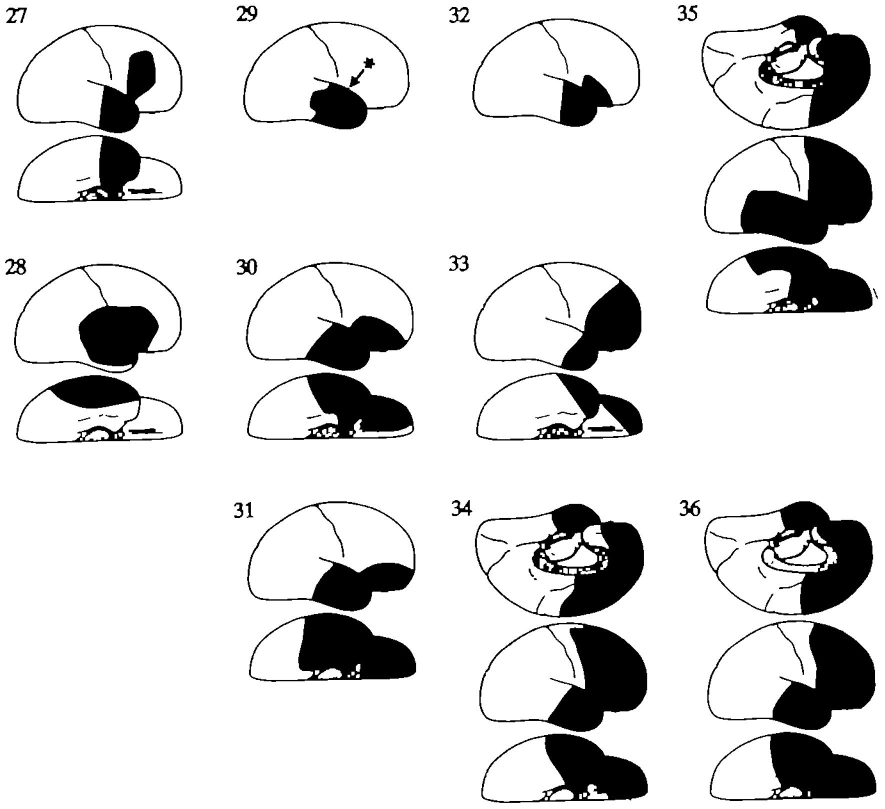

FIG. 3. Right frontotemporal group (RFT). Brain maps indicating estimated extent of removal (*see* fig 1 for explanation of the different views of the brain illustrated in this figure). * The frontal operculum has been removed in Case 29. The excisions included the orbital surface in all but 2 cases (27, 28).

involved different odorants from those used in the discrimination task. Immediately before discrimination testing, all subjects were tested for odour detection thresholds in each nostril using a solution of n-butyl alcohol in distilled water, with a modified method of limits and a two-alternative forced choice task (for full description, *see* Jones-Gotman and Zatorre, 1988*a*). The mean threshold values (molar concentration required to detect the presence of n-butyl alcohol consistently) for each group is presented in Table 3. No significant differences between groups or between nostrils were observed in detection thresholds.

RESULTS

The results are shown in fig. 5. The first analysis examined discrimination scores for each group of subjects for each nostril; patients with lesions within the frontal lobes were divided according to side of lesion, but no further subclassification was made at this point.

A two-way analysis of variance with one between-subject factor (group), and one within-subject factor (nostril tested) showed significant effects of lesion group ($F(6,119) = 3.72$, $P < 0.002$), nostril of presentation ($F(1,119) = 9.17$, $P < 0.003$), and the interaction of the two ($F(6,119) = 2.76$, $P < 0.02$). Orthogonal planned comparisons

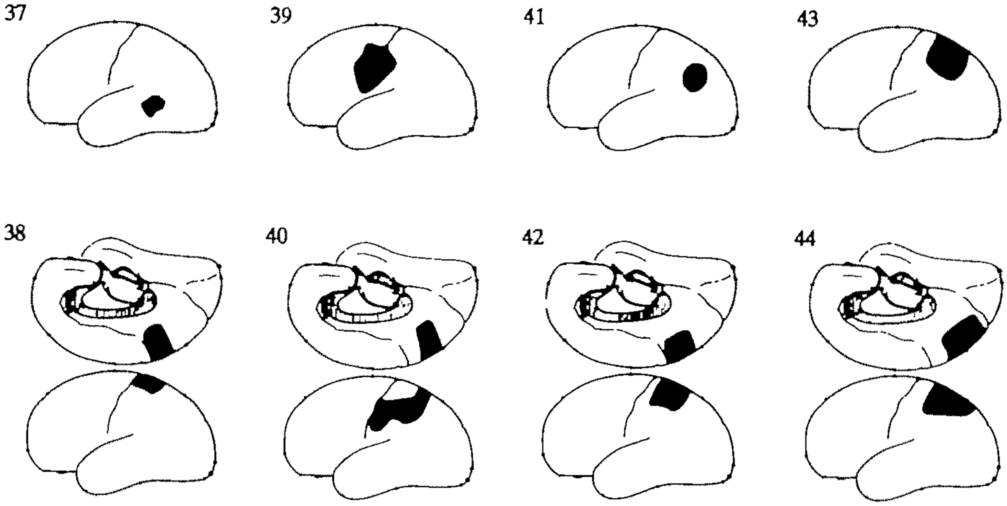

FIG. 4. Left parietal or central group (LP/C). Brain maps showing estimated extent of removal.

TABLE 1 SUBJECT DATA FOR GROUPS STUDIED

Group	*n*	*Sex distribution*		*Mean age (yrs)*	*Mean yrs of education*	*Mean full scale IQ score*
NC	20	11M	9F	25.6	12.8	–
LT	31	18M	13F	25.7	12.3	107
RT	31	14M	17F	29.1	11.3	109
LF	9	5M	4F	27.8	11.4	95
RF	17	12M	5F	33.9	12.0	107
RFT	10	6M	4F	26.5	12.4	102
LP/C	8	3M	5F	30.6	13.1	101

NC = normal controls, LT = left temporal lobe group, RT = right temporal lobe group; LF = left frontal lobe group, RF = right frontal lobe group; RFT = right frontotemporal group; LP/C = left parietal and central regions group.

(Winer, 1971) indicated that all patient groups performed significantly worse overall than the NC group, except for the LP/C group, which did not show any significant impairment. Planned comparisons using the pooled error term also revealed that the interaction with nostril of presentation arises in part from a significant right nostril advantage among the NC group ($P < 0.05$). Furthermore, among the two temporal lobe groups, only performance on the nostril ipsilateral to the lesion was significantly impaired; i.e., the score for the left nostril of the LT group was significantly lower than the left nostril score of the NC group, and the score for the right nostril of the RT group was significantly lower than the right nostril score of the NC group ($P < 0.05$ for all comparisons). Thus the right nostril advantage is exaggerated in the LT group, whereas the RT group shows no difference between the two nostrils. Analysis of the effect of hippocampal excision in the temporal lobe groups did not reveal any additional effect of this variable.

TABLE 2. ODORANT PAIRS USED IN DISCRIMINATION TASK*

Similar pairs	
Gamma-dodecalactone	Aldehyde C-14
Citronellyl acetate	Citronellyl butyrate
Cinnamyl propionate	Cinnamyl butyrate
Lemon extract	Citral rectified
Dissimilar pairs	
Anethol	Benzyl butyrate
Phenylethyl alcohol	Heptanol
Oil of cloves	Allyl sulfide
Butyric acid	Eugenol

* From Mair *et al.* (1980).

TABLE 3 MEAN THRESHOLD DETECTION VALUES*

Group	*Left nostril*	*Right nostril*
NC	3.97×10^{-03}	6.87×10^{-03}
LT	6.10×10^{-03}	1.08×10^{-02}
RT	6.50×10^{-03}	4.04×10^{-03}
LF-Sp	2.70×10^{-03}	7.29×10^{-02}
LF-Orb	5.62×10^{-03}	1.30×10^{-03}
RF-Sp	1.22×10^{-02}	2.35×10^{-03}
RF-Orb	3.70×10^{-03}	3.89×10^{-02}
RFT	3.10×10^{-02}	4.47×10^{-02}
LP/C	7.06×10^{-03}	1.18×10^{-03}

* Molar concentration of n-butyl alcohol.

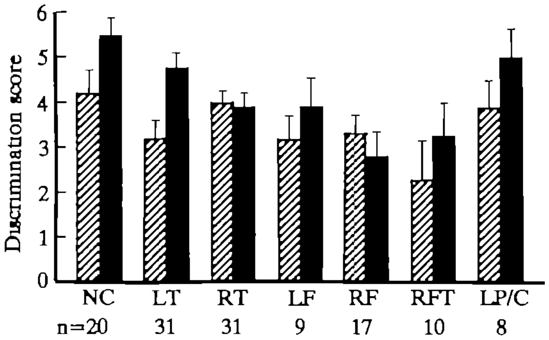

FIG 5 Mean olfactory discrimination score (maximum 8) for left and right nostrils in the 7 groups of subjects studied Error bars indicate 1 SEM Hatched areas = left nostril; filled areas = right nostril For abbreviations, *see* Table 1

The significant impairments in the frontal lobe groups were not quite as systematic. Whereas the RF group showed a significant deficit only in the nostril ipsilateral to the lesion ($P < 0.01$), the LF group's deficit was confined to the nostril contralateral to the lesion (i.e., the right nostril). For the RFT group, whose overall discrimination performance was the poorest of any group, both nostrils were impaired in comparison with normal ($P < 0.01$ in both cases).

In an effort to examine the specific effects of lesions of the orbital surface of the frontal lobe, the patients with frontal lobe excision were subdivided according to the side and site of the damage, yielding four groups: those with LF lesions sparing or including the orbital surface, and those with RF lesions sparing or including the orbital surface. These four groups were included in a subsidiary analysis of variance which also included the NC group. This breakdown of the data is shown in Table 4. The analysis resulted in a significant overall group effect (F(1,41) = 4.90, $P < 0.003$) with no other effects or interactions; only the patients with right orbitofrontal damage were significantly impaired according to post hoc Newman-Keuls tests. Planned comparisons, using the pooled error term, were also run in order to test the hypothesis that only performance in the nostril ipsilateral to the lesion would be impaired by comparison with the normal performance expected in that nostril. These contrasts did not reveal a significant deficit in either of the LF subgroups. However, it is notable that the score for the left nostril in the LF group with orbital damage is poor. Because of the small number of observations in this sample, however, no statistical significance can be attached to this score. The RF group whose lesions spared the orbital surface did show a deficit in the right (ipsilateral) nostril ($P < 0.05$), whereas those patients with RF lesions including the orbital cortex were impaired ($P < 0.01$) in both nostrils (*see* Table 4).

TABLE 4 MEAN OLFACTORY DISCRIMINATION SCORE (MAXIMUM 8) AND SD (BRACKETED) FOR PATIENTS WITH FRONTAL LOBE EXCISION SUBDIVIDED ACCORDING TO ORBITOFRONTAL EXCISION AND FOR NORMAL CONTROL SUBJECTS

Subject group	*n*	*Left nostril*	*Right nostril*
LF-Sp	3	3.67 (1.53)	4.00 (2.65)
LF-Orb	6	3.00 (1.55)	3.83 (1.83)
RF-Sp	9	3.56 (1.59)	3.56 (2.13)
RF-Orb	8	3.00 (1.85)	2.00 (1.85)
NC	20	4.25 (2.00)	5.45 (1.54)

The next analysis was performed to investigate possible sex differences. Because there were insufficient numbers of subjects in the frontal lesion groups to break down by sex, only patients with temporal lobe lesions and controls were included in this analysis. No significant effect of sex was found on the overall discrimination measure. As before, there was a significant interaction of nostril by lesion, but there was also a significant interaction of nostril by lesion by sex (F(2,76) = 7.08, $P < 0.001$). Because this interaction was not predicted, it was broken down by means of Newman-Keuls tests. These showed that the right nostril advantage previously shown in the NC group was present only in male subjects ($P < 0.05$). Also, the large advantage in the

LT group favouring the right nostril was significant only in the female subjects ($P < 0.05$).

Finally, we examined performance as a function of item difficulty, to determine whether the effects observed might be primarily due to poor performance on the more difficult items. For this analysis the stimulus set was divided into similar and dissimilar items according to the data from Mair *et al.* (1980). This yielded a highly significant effect of similarity ($F(1,112) = 121.41$, $P < 0.0001$), but this factor did not interact with any other. Thus performance by all subjects was affected in a like manner by the similarity of the odorants (i.e., similar pairs were much more difficult to discriminate than dissimilar ones).

DISCUSSION

The data from this experiment may be summarized as follows. A consistent difference in olfactory discrimination favouring the right nostril was noted for normal subjects. Lesions to either temporal lobe result in a decrement strictly limited to the nostril ipsilateral to the lesion. Lesions to the frontal lobes also result in severe deficits, particularly when the damage includes the right orbital surface, in which case the deficit appears in both nostrils. This is also the case for combined lesions of the right temporal and orbitofrontal cortices. The data for left frontal lesions are not as clear; they seem to suggest impairments in the right nostril, but given the limited number of subjects with such lesions in our sample, the meaning of this finding remains unclear. The patients in the LP/C group serve as a valuable control, insofar as none of their lesions encroach upon regions thought to be important in olfaction. As predicted, their performance in all cases was within normal limits.

It is important to keep in mind that these patients all demonstrated normal detection thresholds for n-butyl alcohol (Jones-Gotman and Zatorre, 1988*a*), and no differences were found between the thresholds in the two nostrils of any group (*see* Table 3). Thus any deficits uncovered are not attributable to a simple primary loss of olfactory sensitivity; rather, the deficit must lie at a higher level of processing.

Analysis of sex differences did not yield any overall effect of this variable on discrimination performance. Although some authors have found women to be superior to men on certain olfactory tasks (e.g., Doty *et al.*, 1985), the differences tend to be small, and may be obscured in our sample by the larger variability related to the effect of the lesion. The interaction between sex and nostril of presentation is more difficult to interpret. In the normal group, the right nostril difference was primarily found among men; however, this finding would not appear to be important since our recent study with a much larger sample has established that the effect is indeed present to the same degree in both sexes (Zatorre and Jones-Gotman, 1990). The meaning of the interaction among the LT group, in whom the poor left nostril performance was primarily found among the female subjects, remains to be established.

Two of our findings strongly suggest that there is a relative functional asymmetry between the hemispheres in odour discrimination, with a right hemisphere predominance on this task. First is the nostril difference favouring the right side among the normal control subjects, and second is the fact that only RF orbital lesions and RFT lesions yielded consistent impairments in both nostrils. We interpret this latter fact in particular as evidence that the crucial region underlying olfactory discrimination is likely situated in the right orbitofrontal cortex.

Several independent facts support this conclusion, both in terms of localization and lateralization. The suggestion that the orbitofrontal cortex is important in higher-order olfactory function is consistent with both the anatomy and physiology of this region, which receives both direct and indirect olfactory input from the primary olfactory cortex. The elegant studies by Tanabe *et al.* (1975*b*) indicate that neurons in the lateral posterior orbitofrontal cortex (LPOF) are precisely tuned to specific odorants, and they seem to respond differentially to very similar odours; from this we may conclude that LPOF neurons are important in distinguishing among different odours. Such a conclusion is supported by the demonstration that a bilateral LPOF lesion is sufficient to disrupt olfactory discrimination in monkeys (Tanabe *et al.*, 1975*a*), as well as dogs (Allen, 1941) and rats (Eichenbaum *et al.*, 1980). The few human experimental studies examining this issue (Potter and Butters, 1980; Jones-Gotman and Zatorre, 1988*a*) are also consistent with the notion that the orbitofrontal area plays a major role in olfactory processing.

As for nostril asymmetry, the evidence is sparse. Among normal subjects, no previous report, to our knowledge, has documented a difference between the nostrils in olfactory discrimination in the absence of threshold differences. However, we have recently replicated this effect with the same materials in a much larger sample of normal subjects (Zatorre and Jones-Gotman, 1990). The right nostril effect would therefore appear to be quite stable and reproducible.

There have been sporadic reports of differences between the nostrils in detection thresholds among normal subjects, but the conclusions thus far are equivocal: for example, as early as 1900, Toulouse and Vaschide reported an advantage of the left nostril for detecting camphor, but lower detection thresholds in the right nostril for ammonia. More recent studies have also produced contradictory results: one found no significant nostril differences (Koelega, 1979), and another found differences related to handedness, with right-handers more sensitive in the right nostril (Youngentob *et al.*, 1981). Pendse (1987) also reported a right nostril advantage for an intensity scaling task, but only among right-handed women.

Another important point with respect to the nostril differences is that the stimulus pairs used in this study probably have both an olfactory and a trigeminal component. Insofar as the task required a qualitative difference to be registered, the most important information would likely be carried via the first cranial nerve, which projects ipsilaterally. However, it is not possible to be certain that there was no contribution from the (crossed) fifth nerve. If there was a trigeminal component, it would tend to obscure any nostril differences, since it would act in an opposite direction from the asymmetry produced by the olfactory input. Thus the fact that consistent differences were found is all the more remarkable if this is the result of some mixture of both olfactory and trigeminal inputs.

In terms of laterality differences, the data from lesion studies are also not completely consistent, although there is some evidence for right hemisphere lesions resulting in greater impairments. Thus Rausch *et al.* (1977) as well as Abraham and Mathai (1983) and Jones-Gotman and Zatorre (1988*b*) reported significantly greater deficits following right temporal lobe lesions in odour matching and recognition tasks, respectively. On the other hand, neither Eskenazi *et al.* (1983, 1986) nor Jones-Gotman and Zatorre

(1988*a*) reported significant differences related to side of excision in several other olfactory tasks. Mostly the same groups of patients participated in this latter study as in the present one, making the contrast in results more striking. However, the previous study (Jones-Gotman and Zatore, 1988*a*) involved odour identification, which may be a more complex task than quality discrimination in that the subject must successfully match the perceptual experience with information stored in memory. Moreover, identification also involves an important verbal component. These factors may contribute to the bilateral nature of the impairments observed in that study.

Although these conflicting findings are undoubtedly related to differences in stimuli and procedures used between the various studies, it is consistent with the present findings that when differences do emerge either between the nostrils in normal subjects, or between the effects of unilateral lesions, these differences generally favour the right hemisphere. It therefore seems reasonable to conclude, taking together the evidence reviewed above, that the present data may be interpreted as reflecting the greater importance of the right orbital region in olfactory discrimination.

If this conclusion is correct, there are still some outstanding questions, particularly for the results of the LF group. Unfortunately, due to the limited sample size, we are unable to come to a definite conclusion regarding the possible role of the left orbitofrontal cortex. It is the case, however, that when all patients in the LF group are compared with normals, there is an overall deficit, but whether this deficit is confined to one nostril is not clear. It remains for future studies to clarify the specific role of the left orbital cortex in olfactory discrimination.

With respect to the impairments observed in the ipsilateral nostril following temporal lobectomy, there are two possible interpretations of the results. One possibility is that such a lesion disrupts the input to the orbitofrontal cortex from the prorhinal cortex (Potter and Nauta, 1979), and this disconnection accounts for the observed deficit. Another equally plausible interpretation is that at least some processing takes place in the temporal lobe itself, and it is this disruption that leads to the deficit. This latter conclusion is supported by the studies reviewed above that found deficits with unilateral temporal lobectomy, and by the data of Eichenbaum *et al.* (1983) showing an essentially complete inability to distinguish stimuli by smell in patient H.M., who has a bilateral medial temporal lobe resection. Our findings are also consistent with those of Eskenazi *et al.* (1986) who noted that unilateral lesions of the temporal lobe led to deficits confined to the ipsilateral nostril.

Either of these interpretations would be consistent with our primary conclusion that the right orbital cortex is the most important neural substrate for olfactory discrimination. However, it is probable that both the left and the right orbital regions are involved in normal olfactory discrimination, with the difference between them being only relative. This conclusion is reasonable given the substantive evidence from our previous study (Jones-Gotman and Zatorre, 1988*a*) that unilateral lesions to either orbitofrontal region lead to deficits in olfactory identification. It is also consistent with the data from callosotomized subjects (Gordon and Sperry, 1969) in whom the two hemispheres are disconnected. These subjects can perform olfactory matching tasks with stimulation to either nostril, thus demonstrating that both hemispheres are capable of processing some olfactory input. Further study of these callosotomy subjects (Gordon, 1974) suggested a slightly better matching performance when stimuli were presented to the left nostril, but this is difficult to interpret due to possible interference from left hemisphere verbal responses, and also because of possible extracallosal damage. Taking into account all the evidence reviewed above, it would thus appear that the right hemisphere advantage arises only under specific circumstances, perhaps when the task involves a certain type of qualitative judgement.

ACKNOWLEDGEMENTS

We thank Drs A. Olivier, W Feindel, R. Leblanc and J.-G. Villemure for access to their patients and for providing detailed information regarding neurosurgical excisions. We also express our gratitude to the patients who participated in this study for their cooperation. We are also grateful to Drs R. Mair and T. Engen who made available to us the stimuli used in this study This research was supported in part by grant MA-10314 to the authors and MT-2624 to Dr B. Milner from the Medical Research Council of Canada. These results were presented at the annual meeting of the Society for Neuroscience, Toronto, November 1988.

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(*Received July 4, 1989. Revised December 28, 1989. Accepted January 9, 1990*)

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MUSCLE PERFORMANCE, VOLUNTARY ACTIVATION, TWITCH PROPERTIES AND PERCEIVED EFFORT IN NORMAL SUBJECTS AND PATIENTS WITH THE CHRONIC FATIGUE SYNDROME

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SUMMARY

The decrease in maximal force-generating capacity, the degree of central activation of the muscle, and the subjective perception of effort were measured during prolonged submaximal isometric exercise in 12 male patients suffering from the 'chronic fatigue syndrome' and 13 naive, healthy male subjects. Maximal voluntary isometric torque generated by the elbow flexors was measured before, and at 5 min intervals during an endurance sequence of 45 min of repetitive isometric contractions (6 s duration, 4 s rest interval) producing 30% of the initial maximal voluntary torque. Electrical stimuli were also delivered to the elbow flexors to measure the contractile force in the intervals between voluntary contractions. The degree of central motor activation during maximal voluntary contractions was assessed using a sensitive method of twitch interpolation. In addition, the perceived effort required to achieve the target submaximal contractions was recorded using a standardized self-report scale. A high degree of central activation was achieved in maximal contractions during the endurance sequence both in the patients (mean of maximal force 93.6%; SD 7.8%), and in the control subjects (mean 90.9%, SD 9.5%). The relative torque produced by either voluntary or electrically stimulated contractions was not significantly different between patients and control subjects throughout the test. There was no significant difference in the perceived exertion between the patients and control subjects. These findings support the concept that neither poor motivation, nor muscle contractile failure is important in the pathogenesis of 'fatigue' in patients with the chronic fatigue syndrome.

INTRODUCTION

The syndrome of persistent disabling fatigue often occurring after viral or other infection has recently been the focus of renewed research interest. Criteria have been developed for the diagnosis of the disorder (Holmes *et al.*, 1988; Lloyd *et al.*, 1988*b*). It has been suggested that the various names for the syndrome such as postviral fatigue syndrome (Behan *et al.*, 1985), myalgic encephalomyelitis (Anonymous, 1956) and chronic Epstein-Barr virus infection (Straus *et al.*, 1985) be abandoned and the disorder be designated chronic fatigue syndrome (CFS) (Holmes *et al.*, 1988; Lloyd *et al.*, 1988*b*). Although reports in the medical literature have focused on apparent epidemics of the disorder, such as those in the Los Angeles County Hospital (Gilliam, 1938) and the Royal Free

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Hospital, London (Medical Staff of the Royal Free Hospital, 1957), the majority of cases occur sporadically in the community (Behan *et al.*, 1985; Ramsay, 1986; Lloyd *et al.*, 1988*b*).

Profound 'fatigue' after minor physical activity associated with an inability to perform tasks that were previously achieved with ease is the major complaint in patients with the chronic fatigue syndrome. However, there has been little attempt to document objectively this primary symptom of fatigue or to define its pathophysiology. It may reflect a peripheral or a central abnormality. The demonstration of enteroviral RNA in the muscles of 20 of 96 patients (21%) with chronic fatigue syndrome (Archard *et al.*, 1988), and the persistence of enteroviral antigen in the serum of 44 of 87 patients (51%) (Yousef *et al.*, 1988), raised the possibility that persistent infection in the muscles may produce a disturbance of muscle metabolism and hence excessive fatigue. Abnormal intracellular acidosis has been demonstrated in ^{31}P-NMR studies of exercised muscle of a small number of subjects (Arnold *et al.*, 1984; Yonge, 1988). There is considerable evidence against this hypothesis, including the lack of significant abnormalities in light and electron microscopic examination of muscle biopsies (Behan *et al.*, 1985; Byrne and Trounce, 1987). Biochemical analysis of a range of glycolytic and mitochondrial enzymes in muscle and standard electromyography have shown no definite abnormality (Richardson, 1956; Byrne and Trounce, 1987). Single-fibre electromyography has demonstrated no impulse blocking but increased jitter (Jamal and Hansen, 1985). In the absence of impulse blocking (i.e., effective failure of a neuromuscular junction), the electromyographic findings cannot account for the symptom of fatigue unless blocking occurred in high threshold motor units not sampled in the electromyographic studies.

Two studies of muscle performance have shown minimal or no significant abnormality in patients with the chronic fatigue syndrome. In one study, maximal isometric contractions were repeated with a 50% duty cycle, while strength, force decline and recovery were measured (Lloyd *et al.*, 1988*a*). Contractile failure in this type of maximal isometric exercise has been attributed to intramuscular metabolic changes including lactic acidosis and depletion of glycogen and creatine phosphate (Karlsson and Ollander, 1972; Edwards *et al.*, 1975; for review, *see* Edwards, 1981). However, this type of maximal exercise is not common during the normal activities of daily life. In the second study, maximal isometric strength measurements in the patients fell within the authors' normal range except in a minority of subjects who had evidence of impaired central motor drive (Stokes *et al.*, 1988). Electrical stimulation of adductor pollicis showed no differences in the contractile performance and relaxation rates between patients and control subjects (Stokes *et al.*, 1988).

The demands of normal daily activities on skeletal muscles are more closely reproduced by repetitive, submaximal exercise. In contrast to the fatigue produced by repetitive maximal isometric exercise, the decline in peripheral force-generating capacity in submaximal contractions is not initially associated with significant metabolic disturbance (Vøllestad *et al.*, 1988). In this type of exercise, failure of excitation-contraction coupling is likely to be the major cause for the *initial* reduction in force (Vøllestad *et al.*, 1988).

The present study was specifically designed to (1) measure the voluntary performance of *naive* normal subjects and patients with chronic fatigue syndrome in *submaximal* voluntary contractions; (2) quantify the degree of voluntary motor drive during a concomitant series of maximal voluntary contractions using a sensitive version of twitch interpolation (Gandevia and McKenzie, 1988; Hales and Gandevia, 1988); (3) check the subject's report of perceived effort during prolonged submaximal contractions; and (4) compare the above measurements of voluntary and perceived performance with that evoked by electrical stimulation of the muscle. The study was designed to enable collection of all data for each subject in one experimental session, with identical conditions for patients and control subjects.

METHODS

Subjects

Carefully evaluated male patients who fulfilled our diagnostic criteria for chronic fatigue syndrome (Lloyd *et al.*, 1988*b*; Table 1) were the subjects in this study. These criteria highlight the positive features of the syndrome and were used in conjunction with investigations to exclude alternative diagnoses (including blood count and differential count, renal and liver function tests, thyroid function tests, serum creatine kinase estimations, antinuclear antibodies and serological tests for syphilis, hepatitis B and AIDS). To fulfil the criteria, patients were required to have abnormal cell-mediated immunity. The immunological abnormalities were included in these restrictive criteria to add an objective measure to define a more homogeneous patient group for research studies. The conventional neurological examination was normal in all subjects.

TABLE 1 CRITERIA FOR THE DIAGNOSIS OF THE CHRONIC FATIGUE SYNDROME

To fulfil the criteria for chronic fatigue syndrome (CFS), a patient must have no alternative diagnosis reached by appropriate investigations (*see* Methods) and have the following

1 *Chronic persisting or relapsing fatigue* of a generalized nature, exacerbated by minor exercise, causing significant disruption of usual daily activities, present for greater than 6 months.

2. *Neuropsychiatric dysfunction*, including impairment of concentration evidenced by difficulty in completing mental tasks which were easily accomplished before the onset of the syndrome, and/or new onset of short-term memory impairment.

3. *Abnormal cell-mediated immunity* evidenced by a reduction in the absolute count of CD4 and/or CD8 lymphocyte subsets, and/or reduced delayed-type hypersensitivity skin responses.

In addition the following findings are supportive of a diagnosis of CFS.

1 *Symptoms* (persistent for greater than 6 months, with no other cause found on investigation). myalgia, arthralgia, headaches, depression, tinnitus, paraesthesiae, sleep disturbance.
2. *Signs* (present on two or more occasions subsequent to an initial illness). lymphadenopathy, localized muscle tenderness, pharyngitis.

In the 12 patients in the present study, abnormal cell-mediated immunity was indicated by T cell lymphopenia in 4 patients and reduced delayed-type hypersensitivity skin testing in 8 patients. For healthy Australian adults, 1% have reduced delayed-type hypersensitivity skin responses using the identical test to that employed in the assessment of the patients (Frazer *et al.*, 1985). Three of the 12 patients tested had developed the chronic fatigue syndrome after a serologically documented Epstein-Barr virus infection. A viral-like illness had immediately preceded the onset of symptoms in 7 of the 9 remaining subjects. The patients had been symptomatic for a median of 30 (range 15–96) months Six of the 12 patients had been forced to discontinue work because of the chronic disability, the remaining 6 patients had missed more than 30% of their working hours in the preceding 3 months due to this disorder. None of the patients was seeking financial compensation for the illness.

Male control subjects were selected from hospital employees (9 subjects) and from individuals who responded to an advertisement for participation in studies of muscle endurance (4 subjects). All subjects were unfamiliar with muscle strength testing. Control subjects were chosen to provide a group of similar age, height and weight as the patients. The general activity of all subjects during the preceding month was assessed using a questionnaire administered by the experimenters (McKenzie and Gandevia, 1986), and the activity was then graded on a 5 tier scale that included 'sedentary' (score = 0), 'regular light exercise' (score = 2), and 'endurance training for sporting events' (score = 4). Informed consent was obtained from all subjects. The study was approved by the appropriate institutional ethics committee.

Muscle testing

Strength and endurance of the elbow flexors were examined, because established techniques (e.g., Gandevia *et al.*, 1983; McKenzie and Gandevia, 1986, 1987) exist for assessment of this muscle group. The specific muscle group selected was thought not to be critical in view of the generalized nature of the muscle fatigue in patients with the chronic fatigue syndrome (Holmes *et al.*, 1988; Lloyd *et al.*, 1988*b*). Testing was performed in a room at 25° C, with the subject seated and the right arm fixed to a vertical isometric myograph. The forearm was fully supinated, and the elbow was flexed at 90°. Torque (which is directly proportional to force) was measured continuously. Stimuli were delivered transcutaneously to the elbow flexors using a pair of 10×10 mm foil and gauze electrodes, soaked in saline and the covered with electrode gel. The cathode was applied at the location on the muscle surface which produces the largest focal contraction of elbow flexion (via biceps brachii and brachialis). This corresponds to the midpoint between the elbow crease and the bicipital groove at the edge of the anterior deltoid. The anode was placed over the biceps tendon just proximal to the elbow crease. These placements have been used in studies of maximal voluntary endurance of the elbow flexors (McKenzie and Gandevia, 1989). The upper arm was then covered with thin plastic to minimize evaporation. Rectangular pulses of 100 μs duration and 180–240 V were delivered to the muscle from a constant voltage stimulator, singly or in pairs (interstimulus interval 10 ms). With this arrangement, single stimuli produce a twitch contraction of over 10% of the maximal voluntary force and stimulus conditions remain stable for over 3 h (Gandevia, unpublished observations).

Protocol

Initially the manoeuvres were explained and practised for the left elbow flexors. Then the study was undertaken with the right elbow flexors. Before commencing the test exercise (fig. 1), the optimal voltage for the twitch contraction using single and paired stimuli, was tested in the resting muscle. In all subjects, electrical pulses at the selected stimulus intensity evoked maximal twitch contractions. The subjects then made a series of brief (2–3 s), maximal contractions to measure the (unfatigued) muscle strength (fig. 1).

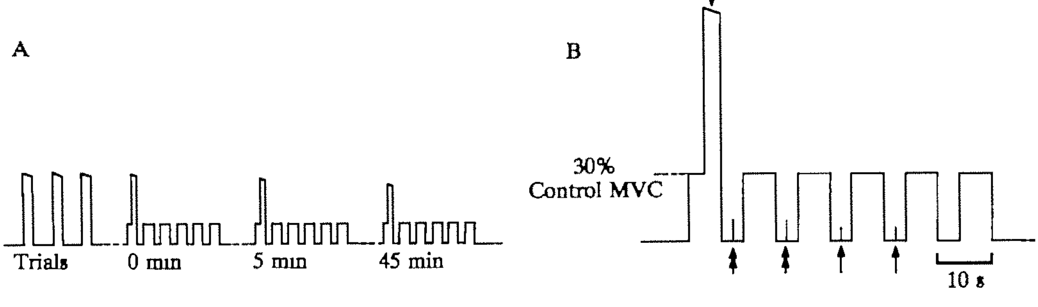

FIG 1. Diagrammatic view of the sequence of contractions A, 3 maximal voluntary contractions were initially performed during which interpolated electrical stimuli to the elbow flexors were delivered ('trials'). Subsequently, the subject began repeated isometric contractions to 30% of the measured maximal voluntary force. Each contraction lasted 6 s with a 4 s rest interval and the sequence continued for 45 min. In the first contraction and then at exactly 5 min intervals the maximal voluntary force was assessed by asking the subject to increase the submaximal force to maximal after 3 s. B, Sequence of events associated with one of the maximal voluntary contractions (MVC) during the exercise sequence. Paired stimuli (interstimulus interval 10 ms, double arrows) were delivered during the maximal contraction, immediately after it and after the next submaximal contraction. The latter stimuli delivered at rest allowed measurement of the potentiated twitch response which served as the reference for any force increments evoked during the preceding maximal voluntary contraction. Single stimuli were also delivered to determine the twitch force and contraction time (single arrows).

During the contractions, twitches were evoked by paired stimuli to test with 'twitch occlusion' whether the muscle was maximally activated by voluntary effort (Merton, 1954; Belanger and McComas, 1981; Hales and Gandevia, 1988; *see* fig. 2). Subjects were required to activate voluntarily more than 95% of maximal elbow torque (*see below* for calculation) in at least one of these trial contractions to proceed further in the study. All patients and control subjects were able to do so (cf Stokes *et al.*, 1988). The target force was then set at 30% of the control maximal voluntary contraction, and this force level was marked on a set of light-emitting diodes in front of the subject. The subject then performed repeated contractions of 6 s duration, separated by 4 s of rest (duty cycle 60%; fig. 1). At the start, and then at 5 min intervals during the 45 min of exercise, the subjects made a brief sustained maximal contraction during the last 3 s of a target force contraction to measure the decline in the maximal voluntary force (fig. 1). A paired stimulus was delivered during each sustained maximal voluntary contraction to measure the adequacy of central motor drive by twitch occlusion. In addition, paired and single pulses were delivered repetitively, between the submaximal contractions (immediately following the maximal voluntary contractions; *see* fig. 1B) to measure the decline in force-generating capacity of the muscle. For each stimulus the amplitude and latency of response were recorded at high gain using a digital oscilloscope with cursors (*see* Hales and Gandevia, 1988). The contraction time was measured from stimulus onset to the peak of the contraction. The temporal resolution was 0.5 ms. The force resolution was 0.057 Nm and 0.028 Nm for the twitch contractions and superimposed twitches respectively. Subjects were given uniform instructions throughout the test, and then vigorously exhorted to perform to their peak for the maximal voluntary contractions with standardized verbal encouragement. Torque was also sampled at 50 Hz by computer and stored for subsequent analysis of endurance performance.

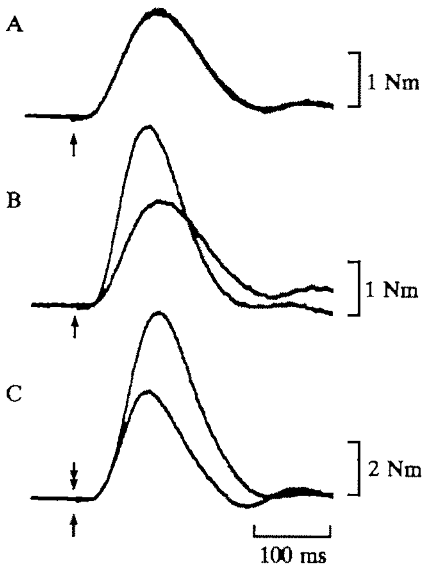

Fig 2. Data from a single subject to demonstrate some of the parameters monitored before and during the exercise sequence A, superimposed twitch responses of elbow flexors to single stimuli of 180 and 200 V delivered through surface electrodes (*see* Methods). The responses do not differ B, 2 responses superimposed to single stimuli to show the potentiation produced by the 3 trial maximal voluntary contractions. C, 2 responses superimposed to show the increase in twitch force produced by the paired stimuli (interstimulus interval 10 ms) compared with the single stimulus. In this and fig. 3, responses are taken from photographs of original oscilloscope records Arrows as in fig. 1.

Five minutes after the commencement of the exercise, and at 5 min intervals thereafter, the subjects rated the degree of perceived effort required to perform the submaximal contractions on a modified Borg scale of perceived exertion (Borg *et al.*, 1985; *see* Appendix). It provides values from 'infinitely small amount' (score = 0) to 'large amount' (score = 5), to 'extremely large amount (maximal exertion)' (score = 10).

Analysis

The degree of motoneuronal drive was measured by amplification of the twitch generated by the stimuli superimposed on the maximal voluntary contraction. With this technique a superimposed twitch generating torque as small as 1–2% of the torque of the maximal twitch can be resolved (Gandevia and McKenzie, 1988; Hales and Gandevia, 1988; *see also* fig. 3). The size of this superimposed twitch contraction was expressed as a percentage of the torque measured in the twitch contraction from the resting muscle immediately following the appropriate maximal voluntary contraction. This was then subtracted from 100% to provide a quantitative measure of the motoneuronal output. It is termed here the degree of 'central activation'.

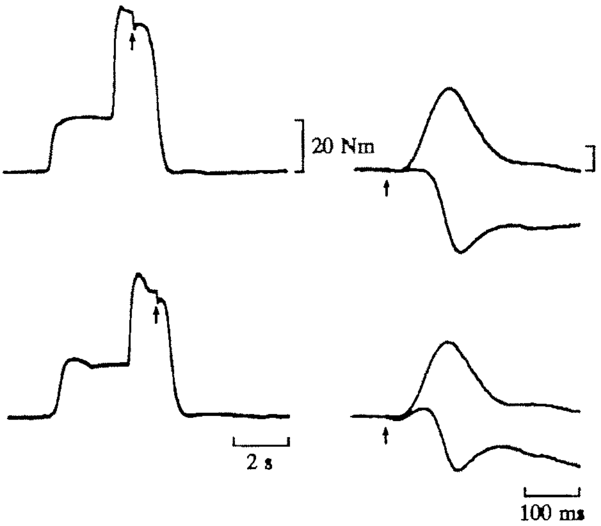

FIG. 3. Data from one subject to show the measurement of the maximal voluntary torque (*left panels*) and the responses to twitch interpolation (*right panels*). Responses in the *upper panels* obtained at the onset of the exercise period and in the *lower panels* 10 min into the exercise. Paired stimuli to the elbow flexors delivered at the arrow. In panels on the right the response to the interpolated stimuli are superimposed on the 'control' response obtained immediately after the contraction. This facilitates the measurement of small evoked increases in force with the correct onset latency. For illustration, the gain for the twitch contraction has been reduced by a factor of 2 compared with that for the interpolated responses. In the sustained maximal contraction shown in the lower panel the subject failed to obtain the maximal output from the stimulated muscles. For details of the calculation of the degree of voluntary activation, *see* text. Vertical calibration (*right panels*) twitch contractions 4 Nm; responses to interpolated stimuli 2 Nm. (During the test, all traces were displayed at higher gain and at a faster sweep speed than depicted here; *see* Methods.)

Differences between the subject groups in age, height, weight and training status, and the initial measures in the unfatigued muscle, were assessed using unpaired two-tailed t tests. To assess progressive changes during the exercise, simple regression analyses were applied to both the group and individual data from patients and control subjects for the degree of central activation, and for measurements of contractile properties. Maximal voluntary torques during the exercise sequence were expressed for each subject as a percentage of the control value. Similarly, the mean of two torque values obtained for each repeated electrically-evoked twitch was expressed as a percentage of the initial value at the onset of the exercise sequence. Two factor (repeated measures) ANOVAs were used to compare the changes in these variables between groups.

RESULTS

The patients and control subjects did not differ significantly in age, height or weight. The patients had a mean age of 34 (SD 11) yrs in comparison with a mean of 36 (SD 13) yrs in the control subjects. The mean height of the patients was 180 (SD 5) cm and of the control group was 177 (SD 10) cm, and the mean weights were 73 (SD 9) kg and 72 (SD 17) kg, respectively. The training status of the control subjects was greater than that of the patients (mean 1.9 vs 1.4; $P < 0.05$), due to the reduced amount of exercise undertaken by the patients. All subjects completed the exercise sequence without undue difficulty, and without complaint of excessive pain during the study or afterwards.

Initial voluntary strength and twitch properties

A summary of the measurements of voluntary performance and contractile properties is given in Table 2. The mean of the maximal voluntary isometric strength of the patients with the chronic fatigue syndrome was 69.1 (SD 13.8) Nm which was not significantly different from that of the control subjects who had a mean of 68.0 (SD 15.3) Nm. These values are similar to those reported for several groups of healthy males, from this laboratory using identical techniques (e.g., Colebatch *et al.*, 1986; McKenzie and Gandevia, 1986; Lloyd *et al.*, 1988*a*). The degree of potentiation produced by the 3 initial maximal voluntary contractions of the resting twitch contraction (fig. 2) was similar in the patients with a mean of 56 (SD 29)%, and control subjects who had a mean of 44 (SD 20)% ($P = 0.3$). The mean twitch contraction time for the unpotentiated muscle was 94 (SD 11) ms for the patients and 98 (SD 15) ms for the control subjects ($P = 0.5$). In all subjects, a large proportion of the elbow flexor muscle group was stimulated by the single shocks applied over the biceps brachii. Thus when the torque generated by the potentiated twitch contraction was expressed relative to the maximal voluntary flexor torque, it represented 14 (SD 4)% in the patients 11 (SD 3)% in the control subjects.

TABLE 2 STRENGTH, ACTIVATION AND TWITCH PROPERTIES IN PATIENTS WITH CHRONIC FATIGUE SYNDROME AND CONTROL SUBJECTS*

	Patients with CFS (n = 12)	*Control subjects (n = 13)*
Maximal voluntary strength (Nm)	69 (14)	68 (15)
Voluntary activation (%)[a] – trials[b]	99	99
– 0 min[c]	95 (7)	94 (8)
– 45 min[c]	92 (6)	87 (7)
Maximal twitch force (Nm) – resting	4.7 (2.1)	3.7 (1.0)
– potentiated	6.9 (2.5)	5.3 (1.5)
Twitch contraction time (ms)[d] – 0 min	84 (13)	85 (13)
– 45 min	80 (14)	81 (16)

* Values expressed as a means with SD in brackets. [a] Activation in maximal voluntary contractions measured by twitch interpolation (*see* Methods). [b] Group mean of the best value in the three initial ('trial') maximal voluntary contractions. [c] Group mean obtained from the first (time 0 min) and last (time 45 min) maximal voluntary contractions (*see* Methods and fig 1) [d] Values for potentiated twitches obtained immediately after the first and last maximal voluntary contractions of the exercise sequence

Changes in voluntary strength and twitch force

During the submaximal isometric exercise the peak force in the maximal voluntary contractions performed at 5 min intervals fell in all subjects to approximately 75% of the control maximum (range 60–94%; *see* fig. 4). The potentiated twitch contractions, evoked when the muscles were relaxed between the voluntary efforts, also fell progressively during the exercise sequence in both patients and control subjects. The relative torque produced by these twitch contractions in response to single stimuli fell to approximately 65% of the initial values (range 21–99%; *see* fig. 5). This pattern of decline in voluntary and twitch-generated force is similar to that reported previously for quadriceps (Bigland-Ritchie *et al.*, 1986*a*, *b*; Vøllestad *et al.*, 1988), soleus and adductor pollicis (Bigland-Ritchie *et al.*, 1986*b*). There was no significant difference between patients and control subjects in the fall in voluntary torque (F = 0.45, P = 0.9; ANOVA), or peak twitch torque (evoked between voluntary efforts; F = 0.31, P = 0.9).

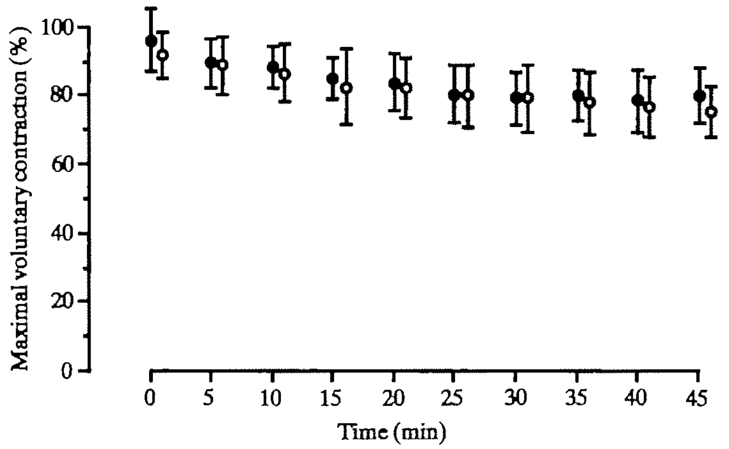

FIG. 4. Maximal voluntary strength of the elbow flexors in 10 static contractions recorded at 5 min intervals during an exercise sequence of 45 min of repetitive submaximal isometric exercise at 30% of the initial maximal voluntary contraction. Each data point represents the mean ±SD for the peak torque attained in each contraction expressed as a percentage of the control maximal voluntary contraction. Data for 12 patients with the chronic fatigue syndrome (closed circles) and 13 control subjects (open circles).

Central activation in maximal voluntary efforts

Six of the 12 patients and 7 of the 13 control subjects were able to achieve 100% activation of the muscle, as demonstrated by the complete absence of additional torque produced by the interpolated twitch (*see* Methods, and fig. 3), in at least 1 of the initial 3 maximal voluntary contractions designed to determine the unfatigued muscle strength. The remaining subjects had interpolated twitch contractions of less than 5% of the torque in the potentiated twitch contraction evoked by paired stimuli to the relaxed muscle, delivered immediately following the maximal voluntary contractions. This indicates a greater than 95% estimated activation of the stimulated muscle in these voluntary efforts prior to the exercise sequence. Indeed, this level of performance (>95% voluntary activation) was a criterion for participation in the study (achieved by all subjects). There was a slight but significant reduction in the degree of central activation during the exercise sequence (fig. 6) in both the patients (r = −0.25, P < 0.01) and control subjects

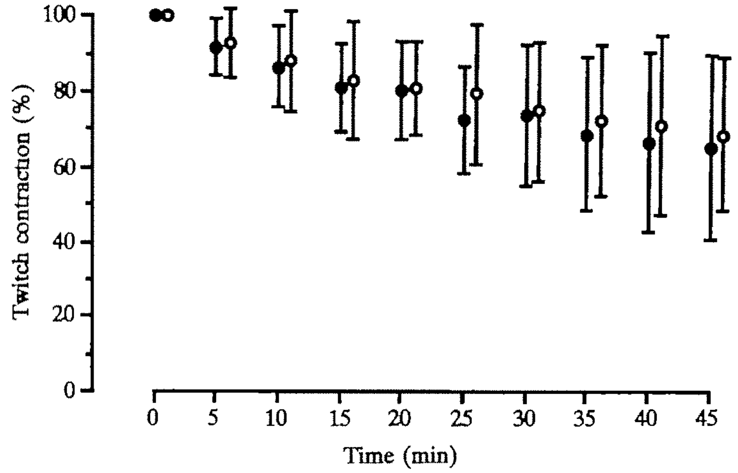

Fig. 5. Potentiated twitch contractions generated by single maximal stimuli to the elbow flexors (*see* Methods), timed immediately after the maximal voluntary contractions at 5 min intervals during the 45 min of submaximal isometric exercise in the patients with chronic fatigue syndrome (closed circles) and control subjects (open circles). Each data point represents the mean ± SD for the torque attained in each contraction expressed as a percentage of the torque in the initial twitch contraction (time 0)

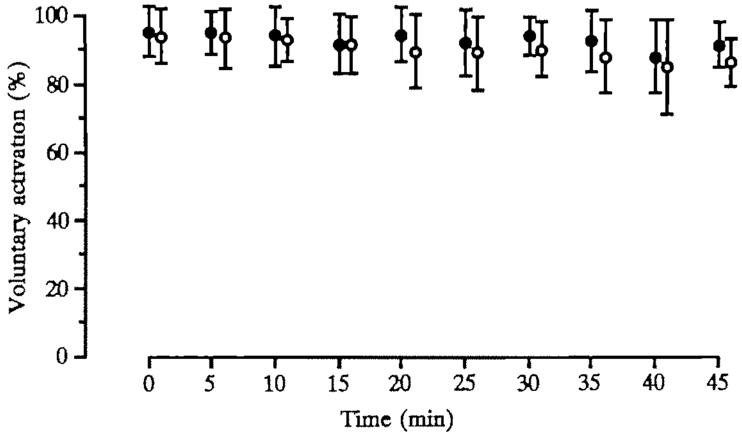

Fig. 6 Voluntary activation of the elbow flexors during *maximal* voluntary contractions recorded at 5 min intervals during an exercise sequence of 45 min of repetitive *submaximal* isometric exercise in patients with chronic fatigue syndrome (closed circles) and control subjects (open circles). The size of the superimposed twitch during a maximal effort is expressed relative to the torque produced in the twitch contraction of the resting muscle immediately following the effort. Thus value is subtracted from 100% to yield a quantitative measure of the voluntary mononeuronal output (*see* Methods). Means ± SD are plotted.

($r = -0.58, P < 0.001$). This trend was statistically significant for 5 individual patients and 6 control subjects. The change in the degree of central activation did not differ between the groups ($F = 0.45, P = 0.91$). Overall during the exercise sequence, the *mean* level of central activation in the grouped analysis of *all* maximal voluntary contractions was slightly greater in the patients than in the control subjects (93.6% (SD 7.8) vs 90.9% (SD 9.5); $P < 0.02$).

Changes in twitch contraction time during the exercise

From the first to the last minute of the exercise sequence, there was a slight reduction in the contraction time of twitches evoked by single stimuli in both the patients (mean reduction 4 ms; $r = -0.3$, $P < 0.01$) and in the control subjects (mean reduction 4 ms; $r = -0.4$, $P < 0.01$). There was no difference in the contraction time after the first maximal voluntary contraction (or the last contraction) between the patients and control subjects (Table 2).

Perceived effort

In the first recording of the perceived exertion on the modified Borg scale, at 4.5 min into the submaximal isometric contractions, the patients mean score of 3.2 (SD 1.4) did not differ significantly from the score of the control subjects who had a mean of 2.6 (SD 0.8). Thus both groups recorded the perceived effort as being approximately 'mild' to 'moderate' after 4.5 min exercise (*see* Appendix). The rating of perceived effort at the completion of the submaximal contractions had risen to a mean of 6.5 (SD 2.5) in the patients and to 5.6 (SD 2.0) in the control subjects, equivalent to a 'large' to 'very large' level of perceived exertion required to complete each submaximal contraction. The change in this score during the exercise sequence was not significantly different between the patients and control subjects ($F = 0.33$, $P = 0.9$).

DISCUSSION

This study documents normal maximal voluntary strength and endurance in patients with the chronic fatigue syndrome in repetitive submaximal exercise. This type of exercise resembles the demands put on the muscle in the usual daily activities, in which patients with the chronic fatigue syndrome experience disabling subjective 'fatigue'. The finding of apparently normal maximal isometric strength in the unfatigued muscle has been previously documented (Lloyd *et al.*, 1988*a*; *see also* Stokes *et al.*, 1988).

Repeated *maximal* isometric contractions in patients with the chronic fatigue syndrome do not produce premature or excessive loss of force in the majority of subjects (Lloyd *et al.*, 1988*a*). Furthermore, in this study, the patients' muscle performance was not significantly impaired when tested 4 h after the initial exercise sequence, at a time when subjective fatigue has typically become a prominent symptom. The metabolic changes produced by such maximal isometric exercise, including intramuscular acidosis and depletion of energy substrates (Karlsson and Ollander, 1972; Edwards *et al.*, 1975; for review, *see* Edwards, 1981) cannot be occurring either prematurely or excessively in patients with the chronic fatigue syndrome. Thus metabolic factors within the muscle are unlikely to play a significant role in the pathogenesis of fatigue in patients with chronic fatigue syndrome.

The specific pattern of *submaximal* isometric exercise used in this study has been previously evaluated for quadriceps femoris in healthy subjects (Bigland-Ritchie *et al.*, 1986*a*; Vøllestad *et al.*, 1988), and produced a similar decline in maximal voluntary force to that documented here. Muscle biopsies taken after 30 min exercise did not show any significant change in the concentrations of creatine phosphate, ATP, lactate, or glycogen. This excluded depletion of energy substrates and/or lactate accumulation as the basis for the concomitant contractile failure. In addition, given that the degree of central activation during the maximal efforts was said to be unchanged in the subjects of this study, the loss of voluntary capacity to generate force was attributed to disturbed excitation-contraction coupling.

The emphasis on abnormalities in cell-mediated immunity as a diagnostic criterion for patients in this study is based on a controlled investigation of immunity in 100 patients with symptoms of chronic fatigue and 100 age and sex-matched healthy adults (Lloyd *et al.*, 1989; *see also* Murdoch, 1988). Given that cytokines such as alpha interferon are released in response to exercise (Viti *et al.*, 1985), and produce 'fatigue' and neuropsychiatric symptoms (McDonald *et al.*, 1987), it has been hypothesized that immunological disturbances may be relevant to the pathogenesis of the chronic fatigue syndrome (Wakefield and Lloyd, 1987).

The findings of this study do not support the possibility that failure of the link between motor drive and the metabolic mechanisms of the muscle (including excitation-contraction coupling) is the site of the pathophysiological disturbance producing fatigue in patients with the chronic fatigue syndrome. Muscle performance in response to both voluntary and electrically-induced contractions was similar in patients with the chronic fatigue syndrome and control subjects. The demonstration of enteroviral RNA in the muscles of patients with the chronic fatigue syndrome (Archard *et al.*, 1988) is therefore unlikely to be of pathophysiological significance in this disorder. Furthermore, the lack of a significant difference between our patients and control subjects in the twitch contraction time during the exercise sequence argues against the significance of excessive intracellular acidosis detected in some subjects with the chronic fatigue syndrome using nuclear magnetic resonance (Arnold *et al.*, 1984; Yonge, 1988).

The degree of central activation of the muscle is affected by several factors including motivation, possible inhibition via nociceptor and other afferents arising in the muscle or joints (Stokes and Young, 1984; Rutherford *et al.*, 1986; Gandevia and McKenzie, 1988). Afferent activity arising from the contracting muscle itself may also cause reflex inhibition during activity (Woods *et al.*, 1987). The accurate measurement of the twitch produced by the stimuli superimposed on the maximal voluntary contractions allowed estimation of the degree of central activation in the subjects in this study. None of the subjects complained of pain in the muscle or joints during the study period. The patients with the chronic fatigue syndrome maintained a similar or slightly greater degree of central activation than the control subjects in the exercise sequence. Lack of motivation therefore played no significant role in the loss of force-generating capacity in the patients of this study. By contrast, a previous study of central activation in patients with the chronic fatigue syndrome (Stokes *et al.*, 1988) suggested that central activation was submaximal in some cases. However, the patients involved in that study were not selected on the basis of established diagnostic criteria and were not asked to undergo a prolonged test of performance.

Judgements about perceived force and effort are biased by signals of centrally generated motor command rather than peripheral signals of force or tension (McCloskey *et al.*, 1974; Gandevia and McCloskey, 1977; for reviews, *see* McCloskey, 1981; Gandevia, 1987). Thus the perceived force increases with muscle fatigue (*see also* Jones and Hunter, 1983), and should increase more rapidly when fatiguability is excessive. The subjects' perception of the effort required to achieve the submaximal force in this study was similar in the patients and control subjects. This finding suggests that the subjective complaint of fatigue *during* exercise in patients with the chronic fatigue syndrome is not due to a disordered central mechanism producing a disproportionate increase in the perceived signals of motor command when there is a (normal) decline in force-generating capacity.

One of the major findings of this study is that the essential symptom of 'fatigue', which provides the basis for one current nomenclature for the disorder, cannot be equated with failure of contractile force. Perhaps surprisingly, there is also no evidence that the centrally generated motor commands associated with the 'sense of effort' increase abnormally in the patients, even during a prolonged period of exercise. The crucial pathophysiological abnormality responsible for the prominent subjective fatigability must therefore lie within the central nervous system above the level of the motor cortex. Indeed, the prevalence of psychological symptoms in this disorder (Wessely and Powell, 1989; Hickie *et al.*, 1990) supports our view that the abnormality involves some higher level function rather than the executive pathways for movement.

ACKNOWLEDGEMENTS

This work was supported by the National Health and Medical Research Council of Australia. We are grateful to Professors D. Burke, J. W. Lance and D. Wakefield for comments on the manuscript.

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(Received July 20, 1989. Revised December 19, 1989. Accepted January 9, 1990)

APPENDIX

Scale of perceived exertion (modified from Borg et al., 1985)

The subjects were asked to describe the amount of effort required to achieve the desired force, using the written scale given below. The standardized verbal instruction to the subjects described a score of 0 as being a 'barely detectable' amount of effort, and a score of 10 as being the 'absolute maximum effort at which you will feel nearly dead!'

Amount of effort	*Score*
Extremely large amount (maximal)	10
	9
	8
Very large amount	7
	6
Large amount	5
Considerable	4
Moderate	3
Mild	2
Minimal	1
Infinitely small amount	0

Brain (1991), **114**, 99–115

SEQUENCING IN PARKINSON'S DISEASE

ABNORMALITIES IN PROGRAMMING AND CONTROLLING MOVEMENT

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SUMMARY

Central programming deficits in Parkinson's disease (PD) were studied in two reaction time (RT) experiments. In Experiment 1, PD patients and controls performed sequences of hand postures that varied in length, the number of different postures (repetitive vs heterogeneous), and the delay interval before movement. Before movement, the PD group planned repetitive movements like controls whereas for heterogeneous sequences RT increased less with sequence length for the PD group, implying less preprogramming. The interresponse time (IRT) data from repetitive sequences showed that the PD group had difficulty controlling movement such that IRTs were faster when sequences were longer, thus allowing more time to schedule the termination of the sequence during the course of movement. For heterogeneous sequences, the PD group made more errors and were slower than controls when changing hand postures, suggesting a deficit in switching between different responses. While RT decreased with a longer delay similarly for both groups, IRT_1 continued to improve only for the PD group but similarly for both types of sequences, suggesting a deficit specific to programming the first response. In Experiment 2, subjects made decisions about the number of different hand postures contained within a sequence. PD patients' decision times improved more with a longer delay only for heterogeneous sequences, suggesting a problem in identifying the number of different hand postures. The results have implications for levels of motor dysfunction in PD which emphasize the influence of sequence length and complexity.

INTRODUCTION

Part of the motor problem observed in Parkinson's disease (PD) has been attributed to central programming deficits although explanations for these deficits are disputed. The present study examines whether abnormalities in programming are found both before and during the execution of hand posture sequences, and whether such deficits vary depending on the complexity of sequences.

Early evidence suggested that once a predictable movement was initiated, PD patients could carry out the motor program if the movement was externally guided (Bloxham *et al.*, 1984; Day *et al.*, 1984). Some studies have shown that PD impairs the ability to use advance information to initiate and select movements (Bloxham *et al.*, 1984; Sheridan *et al.*, 1987; Pullman *et al.*, 1988), but others have found no such deficit (Rafal *et al.*, 1984, 1987; Stelmach *et al.*, 1986). Discrepant findings concerning the nature of cognitive deficits in PD are due in part to the level of analysis. Abnormalities are not always seen when the amount of subject-initiated organization is minimized,

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such as when subjects are highly practised (Stelmach *et al.*, 1986), when the same response is repeated on successive trials (Rafal *et al.*, 1984), or when movement is externally guided (Bloxham *et al.*, 1984; Day *et al.*, 1984). When subjects must independently construct an internal motor representation to guide movement, cognitive deficits seem to be more evident (Flowers, 1978; Stern *et al.*, 1983, 1984). Such deficits are also evident in motor learning (Harrington *et al.*, 1990) where it is important to independently develop strategies that will improve performance. Motor planning in PD has been less well studied using tasks that require more organizational processing such as the sequencing of movements. Although one study of finger sequencing (Rafal *et al.*, 1987) found that PD patients used advance information to retrieve subprograms from a motor program, which was presumably constructed before the RT interval, others have shown that PD patients do not evidence normal programming of repetitive finger key presses (Stelmach *et al.*, 1987). Programming deficits also have been reported in PD during the sequencing of two distinct movements; the duration of individual movements was longer when performed sequentially than in isolation for PD patients only, suggesting a deficit in switching from one motor program to another (Benecke *et al.*, 1987*a*, *b*).

Although there is consensus that PD patients can construct a motor program, it is not clear whether the output from motor programs is qualitatively similar to that of normals or whether motor programs are optimally used during movement. Recent data showing PD patients can learn a motor task such as rotary pursuit but the amount of learning is reduced (Harrington *et al.*, 1990) suggest they may not make normal use of motor programs even when movement is externally guided and predictable. These issues can be addressed by examining programming processes before and during more complex movements such as sequencing where an internal motor representation must be independently assembled and utilized to control multiple movements.

The purpose of the present experiments was to improve understanding of cognitive deficits in PD as they relate to planning motor sequences independent of purely motor factors. The first objective of Experiment 1 was to determine whether motor deficits in PD are due to a difficulty in using advance information about sequences to improve initiation. If this is the case, RTs should not improve as much for PD patients as for controls when they are given more time to plan the sequence. Secondly, the effect of sequence complexity on PD patients' ability to plan sequences prior to movement was examined by contrasting RT performance on sequences containing different (heterogeneous) or the same (repetitive) hand postures. If PD patients can utilize advance information to plan repetitive sequences they should show no effect of sequence length on RT, similar to normal controls (Harrington and Haaland, 1987), suggesting that repetitive movements are planned as a unit. As for heterogeneous sequences, RT increases with sequence length for controls as they must assemble a motor program containing different subprograms, one for each response in the sequence. If PD patients evidence difficulty constructing a motor plan for heterogeneous sequences, sequence length should have less of an effect on RT in comparison with controls as they may not be programming information about all responses in the sequence. Difficulty using motor plans to control the execution of hand posture sequences was examined by comparing groups on the pattern of interresponse times (IRTs). If PD patients plan repetitive sequences as a single unit and use the output from the motor plan optimally, IRTs should not be affected by sequence length. For heterogeneous sequences, PD patients may have difficulty switching between motor programs which will increase the duration of IRTs, particularly those that require changes in hand postures. Experiment 2 examined whether programming difficulties found for PD patients in sequencing were in part related to problems determining the number of different types of hand postures contained within a sequence.

EXPERIMENT 1

Methods

Subjects

Twenty-four patients with Parkinson's disease (PD) and 20 neurologically intact control subjects were studied. PD patients and controls were right-handed and matched for age and educational level (*see* Table 1). The PD group consisted of 25% females and the control group had 53% females but sex was not related to performance on any of the experimental tasks. The average duration of PD was 6 yrs (SD = 7) and the mean age of onset was 60 yrs (SD = 8). All but one PD patient were being treated with dopaminergic drugs.

TABLE 1 DEMOGRAPHICS AND PERFORMANCE OF CONTROL AND PARKINSONIAN SUBJECTS ON ANCILLARY TESTS

	Control group			*Parkinsonian group*		
	Mean	*SD*	*Range*	*Mean*	*SD*	*Range*
Age	65	8	51–80	66	6	54–77
Education	12	2	8–15	12	2	4–15
Information (WAIS-R)[1]	12.6	2	9–16	11.4	3	5–18
Mini-Mental State	29.4	1	27–30	27.7*	2	25–30
Block Design (WAIS-R)[1]	8.7	2	4–13	6.4*	2	4–11
Line Orientation[2]	27.7	4	19–33	24.3*	5	16–32
Wechsler Memory Quotient	122.6	12	99–143	105.2*	15	76–137
Beck Depression Inventory[3]	5.5	4	0–14	11.7*	7	0–29
NYU Disability Scale[4]				19.8	16	1–51

* $P < 0.01$ for t tests comparing controls and PD subjects. [1] Scale scores (mean = 10±3) [2] Scores corrected for age (scores less than 20 are considered impaired). [3] Scores greater than 9 are indicative of depression. [4] Scores are sums across all items with a score of zero reflecting no disability and a score of 100 representing complete disability. Scores of 40 or greater suggest considerable disability

A board-certified neurologist assessed parkinsonian status using two different instruments. On the Hoehn and Yahr (1967) severity scale 37% were classified in Stage 1 (mild, unilateral involvement), 17% in Stage 2 (bilateral involvement), 42% in Stage 3 (mild to moderate gait disturbance), and 4% in Stage 4 (marked gait disturbance). On the New York University (NYU) Disability Scale (Lieberman, 1974) most patients showed no rigidity (71%), tremor (54%), dyskinesia (96%), postural abnormality (63%), or gait problems (67%). For bradykinesia, 54% of the patients showed impaired motor speed and 83% showed impaired amplitude of movement. Five patients received total scores of 40 or greater on this scale which reflected considerable disability (*see* Table 1).

Neuropsychological data are described in Table 1. There were no significant differences between the groups on the Information subtest from the Wechsler Adult Intelligence Scale-Revised (WAIS-R) (Wechsler, 1981). Although the PD group performed more poorly on the Mini-Mental State Examination (MMS)

(Folstein *et al.*, 1975) (F(1,41) = 13.6, $P < 0.001$), all scores were within the normal range. The PD group also performed more poorly on Judgement of Line Orientation (Benton *et al.*, 1983) (F(1,41) = 6.7, $P < 0.025$), Block Design Subtest on the WAIS-R (F(1,41) = 14.9, $P < 0.001$), and on all subtests of the Wechsler Memory Scale (Wechsler, 1945) except Orientation (F(1,41) = 16.3, $P < 0.001$ for the Memory Quotient). An examination of the distributions of these scores showed that 2 PD patients (8%) scored more than 2 SDs below the control group mean on all three of these tests, 3 (13%) did so on two of these tests, and 5 (21%) performed in this range on only one of these tests. This suggests patients were not generally impaired on all tests although PD patients as a group demonstrated mild cognitive deficits. On the Beck Depression Inventory (Beck *et al.*, 1961), the PD group was more depressed than controls (F(1,41) = 10.3, $P < 0.01$).

Procedures

The experimental task required subjects to execute sequences of hand postures. Fig. 1 is a diagram of the apparatus which was interfaced with a computer and contained a row of 5 vertical plates which required contact with the side of the hand, a row of 5 recessed buttons which required contact with the index finger, and a row of 5 handlebars which required the 4 fingers to wrap around the bar from underneath. Subjects wore gloves equipped with metal contacts The start plate was located to the left of the manipulanda, and subjects always moved from the left to the right using their right hand. When a change in hand posture was made, subjects moved to the right diagonally (up or down) to the next manipulandum. A monitor presented pictorial displays of the motor sequences that described the type of manipulandum associated with each response and its location on the apparatus.

Subjects started each trial by resting their index finger on the start plate which caused a pictorial display of the sequence to appear on the monitor. After a random delay of 250 ms or 2000 ms, a tone signalled

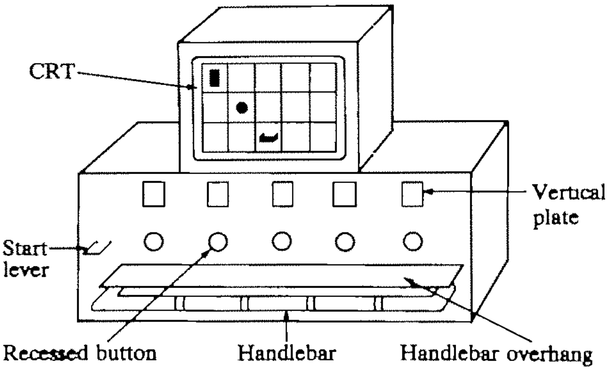

FIG. 1. Diagram of the hand posture sequencing apparatus. A monitor (cathode ray tube, CRT) presented pictorial displays of the sequence

TABLE 2 HAND POSTURE SEQUENCES FOR EXPERIMENT 1*

	Sequence length				
Sequence type	*1*	*2*	*3*	*4*	*5*
Repetitive	P	PP	PPP	PPPP	PPPPP
	B	BB	BBB	BBBB	BBBBB
	H	HH	HHH	HHHH	HHHHH
Heterogeneous		PB	PBP	PBPP	PBPPP
		HB	HBH	HBHH	HBHHH

* The letters P, B, and H designate plate, button, and handlebar responses.

subjects to begin the sequence. On completion of the last response in the sequence, the visual display terminated. Reaction time (RT) was measured from the onset of the imperative stimulus to when subjects lifted their finger from the start plate. The first interresponse time (IRT$_1$) was measured from the end of the RT interval to the completion of the first response (i.e., contact with manipulandum), and subsequent IRTs were measured from the completion of one response to the completion of the next response. An error trial was recorded when subjects took longer than 2000 ms to initiate the movement or to execute a single hand posture or if they executed the wrong hand posture.

Table 2 presents the two types of sequences (repetitive or heterogeneous) which were blocked. Block order was randomized across subjects. Each of the 23 different motor sequences was presented 8 times across blocks in a random order. If subjects made an error, the trial was repeated randomly at the end of the block of trials. Subjects were required to complete correctly 2 practice trials of each of the 23 sequences.

Results

The analyses used a mixed model design with group as the between subject factor and posture, delay interval, and sequence length as the repeated factors. Trend analyses were performed on effects involving sequence length Separate analyses of variance (ANOVAs) were conducted for each measure. Repetitive and heterogeneous sequences were analysed separately.

Repetitive sequences

Errors. There were no differences between groups in the percentage of errors before the imperative stimulus (4%) or the percentage of errors during movement (1%).

Reaction time. The PD group did not show slower RTs, and RT improved as much for the PD group as for controls with a longer delay interval ($F(1,41) = 102.6$, $P < 0.0001$) (Table 3). Fig. 2A shows that sequence length did not interact with group ($P > 0.05$), suggesting that both groups programmed repetitive sequences similarly. Pooling across groups, RT varied with sequence length ($F(4,164) = 4.1$, $P < 0.01$), but this was due to faster RTs for isolated hand postures ($F(1,42) = 7.1$, $P < 0.01$) relative to sequences of postures regardless of delay interval. For both groups, the type of hand posture interacted with delay interval ($F(2,82) = 9.8$, $P < 0.001$) so that with a 250 ms delay, RTs were longer for button (mean = 530) than plate (mean = 514) or handle responses (mean = 494) ($F(2,84) = 14.8$, $P < 0.001$); at the longer delay interval RT did not vary with type of hand posture. This finding shows that the PD group did not show any deficits in constructing a motor program for specifying muscle groups.

Interresponse times. Table 3 shows that for IRT$_1$, the performance of the two groups varied with delay ($F(1,41) = 5.1$, $P < 0.05$) so that while IRT$_1$ was slower for the PD group regardless of delay interval ($F(1,41) = 7.0$, $P < 0.01$), it improved with a longer delay interval for the PD group only ($F(1,23) = 21.6$,

TABLE 3. MEAN (SE) REACTION TIME AND IRT$_1$ (ms) AS A FUNCTION OF DELAY INTERVAL

	Controls			*Parkinsonians*		
	Delay interval (ms)			*Delay interval (ms)*		
	250	*2000*	*Difference*	*250*	*2000*	*Difference*
Repetitive						
Reaction Time	485	336	149*	534	360	174*
	(33)	(13)	(24)	(32)	(17)	(21)
IRT$_1$	523	503	20	664	606	58*
	(30)	(28)	(10)	(36)	(33)	(13)
Heterogeneous						
Reaction Time	492	353	139*	561	379	182*
	(32)	(15)	(19)	(28)	(20)	(19)
IRT$_1$	576	557	19	756	691	65*
	(30)	(28)	(10)	(40)	(38)	(14)

* $P < 0.01$ for the difference between the 250 ms and 2000 ms delay intervals.

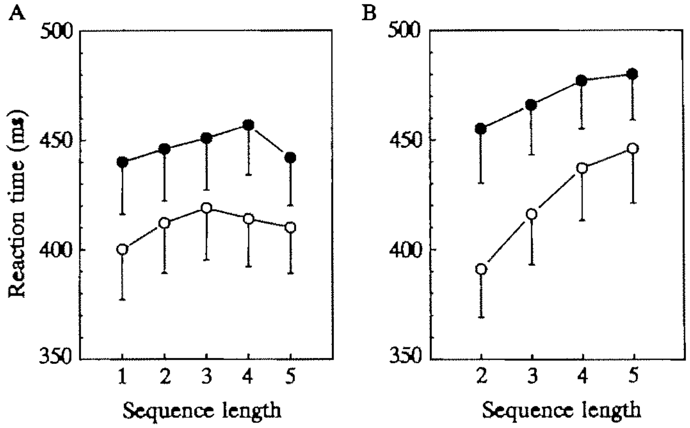

FIG. 2. Reaction time as a function of sequence length. A, mean reaction times with SE bars for repetitive sequences averaged over delay interval. B, mean reaction times with SE bars for heterogeneous sequences averaged over delay interval. Filled circles = parkinsonian patients; open circles = controls.

$P < 0.0001$). For both groups delay interval did not affect IRT_2 through to IRT_5 or reduce the effect size of sequence length on IRT_1.

Fig. 3 shows that the two groups also performed differently as a function of sequence length ($F(4,164) = 7.3, P < 0.001$). Although it took longer for PD patients to execute the first response regardless of sequence length ($F(1,41) = 10.0, P < 0.01$), IRT_1 varied with sequence length for the PD but not the control group (*see* fig. 3A) such that the first hand posture was executed faster as sequence length increased ($F(1,23) = 35.5, P < 0.001$ for linear trend). Similarly, figs 3B and C show that for IRT_2 and IRT_3 group also interacted with sequence length ($F(3,123) = 3.2, P < 0.05$ and $F(2,82) = 5.4$, $P < 0.01$) such that for controls there were no sequence length effects whereas for the PD group the time to execute the second and third posture decreased as sequence length increased ($F(1,23) = 22.3$, $P < 0.001$ for linear trend and $F(1,23) = 6.6, P < 0.05$ for quadratic trend of IRT_2; $F(1,23) = 23.1$, $P < 0.001$ for linear trend of IRT_3). However, fig. 3D shows that for IRT_4, execution time was longer for sequences of 4 than 5 responses for both groups.

There were no overall differences between groups in the execution of the second and third responses ($P > 0.05$); thus, once PD patients initiated a sequence of repetitive movements, execution time was not impaired, at least for the first few movements. However, for IRT_4 and IRT_5 PD patients showed significantly slower execution times than controls ($F(1,41) = 6.0, P < 0.025$ and $F(1,41) = 8.7$, $P < 0.01$, respectively).

Heterogeneous sequences

Errors. The percentage of errors before the imperative stimulus was approximately 4% for both groups, but the PD group made significantly more errors during movement (mean = 5.4% vs 1.2%) ($F(1,41) = 12.8, P < 0.001$). Further analyses showed that the PD group made significantly more errors on the first, second and third responses of the sequence when changes in hand postures were required ($F(1,41) = 10.6, P < 0.01$; $F(1,41) = 6.6, P < 0.025$; and $F(1,41) = 7.9, P < 0.01$), but no differences were seen on the fourth and fifth responses which required repetitions of the same hand posture.

Reaction time. The PD group did not show slower RTs for heterogeneous sequences, and RT improved for both groups with a longer delay interval ($F(1,41) = 142.3, P < 0.0001$) (*see* Table 3). Although it appears that the performance of the PD group improved more than that of the controls with a longer delay interval, this effect was not significant. Delay interval interacted with sequence length ($F(3,123) = 11.1$, $P < 0.001$), showing that RTs for both groups did not increase as much with sequence length (although they still increased) at a longer delay interval. This finding demonstrates that for both groups, more programming is completed with a longer delay interval.

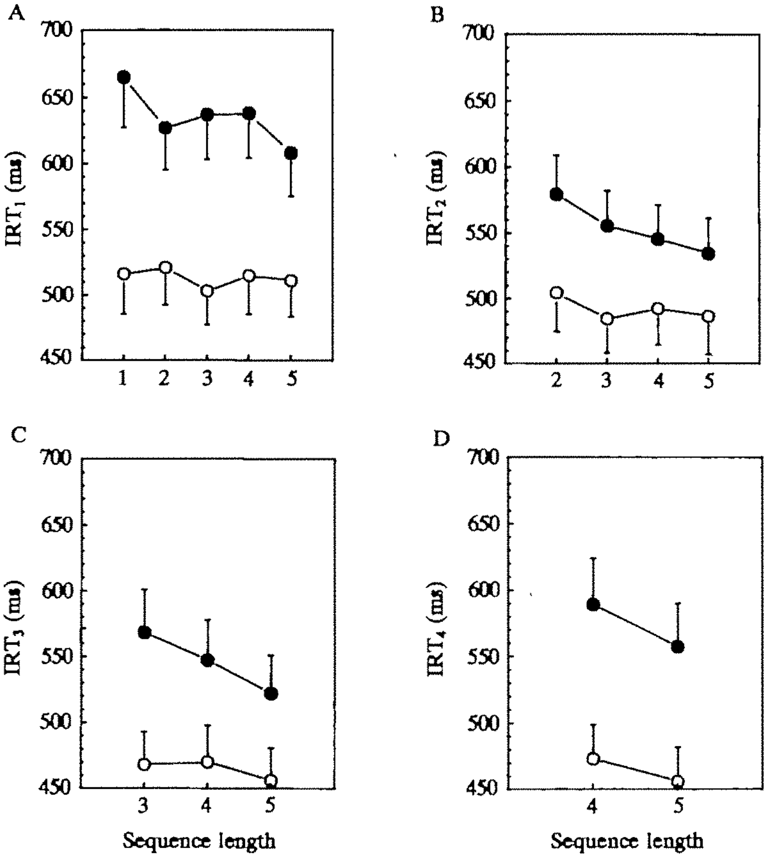

FIG. 3. Interresponse times (IRTs) for repetitive sequences as a function of sequence length. A, B, C and D designate the mean IRTs with SE bars for IRT_1, IRT_2, IRT_3 and IRT_4, respectively. Symbols as in fig 2.

Fig. 2B shows that for heterogeneous RT, group interacted with sequence length ($F(3,123) = 3.4$, $P < 0.025$) such that for control subjects RT increased from sequence lengths of 2 to 4 and became asymptotic thereafter ($F(1,18) = 46.1$, $P < 0.001$ for linear; $F(1,18) = 16.2$, $P < 0.001$ for quadratic trend). For the PD group, only a small effect of sequence length was found, with RTs being faster for sequences containing 2 postures than those containing 4 or 5 postures ($F(1,23) = 8.6$, $P < 0.01$). There was no significant interaction of group by sequence length by delay, indicating that the smaller effect of sequence length on RTs for the PD group relative to controls was found at both delay intervals despite the finding that sequence length had less of an effect on RT with a longer delay interval for both groups.

Interresponse times. Similar to the findings for repetitive sequences, Table 3 shows that for IRT_1 delay interval interacted with group ($F(1,41) = 6.4$, $P < 0.025$) such that the execution of the first posture was faster with a longer delay interval for the PD ($F(1,23) = 22.3$, $P < 0.001$) but not control group. For both groups, delay interval did not affect IRT_2 through to IRT_5 or reduce the effect size of sequence length on IRT_1.

Fig. 4 presents the IRT data for both groups as a function of sequence length. For all IRTs, the PD group was significantly slower than control subjects regardless of sequence length ($P < 0.025$). In addition, the duration of the first IRT increased with sequence length ($F(1,41) = 22.7$, $P < 0.001$ for linear trend) similarly for both groups (*see* fig. 4A) as programming that began during the RT interval is ongoing. While this finding implies that both groups are engaging in similar processes during the execution of the first response, the higher error rates for the PD group during IRT_1 indicates otherwise, and may suggest a speed-accuracy trade off. Fig. 4B shows that no sequence length effects were found for IRT_2 for either group, but for IRT_3 and IRT_4 figs 4C and D show that the performance for the two groups interacted with sequence length ($F(2,82) = 5.3, P < 0.01$ and $F(1,41) = 4.1, P < 0.05$) such that there was no length effect for control subjects, but IRT_3 and IRT_4 decreased with sequence length for the PD group ($F(1,23) = 12.0, P < 0.01$ and $F(1,23) = 12.7, P < 0.01$ for the linear trends). Recall that the third response involves a change in hand posture and subsequent responses are repetitions of this posture which may explain why this pattern of findings is similar to those reported for repetitive sequences.

Mild versus advanced PD

The effect of disease severity was examined by comparing sequencing performance of patients with mild symptoms and those with more advanced symptoms of tremor, rigidity, and bradykinesia. PD patients with ratings on the NYU Disability Scale in the lower (scores less than 11) and upper (scores greater than 20) 38th percentile were compared. There were no differences between the groups in age, disease duration, age of disease onset, amount of tremor and rigidity, or performance on neuropsychological tests. The advanced PD group showed more severe bradykinesia ($F(1,16) = 17.4, P < 0.001$), and scored somewhat

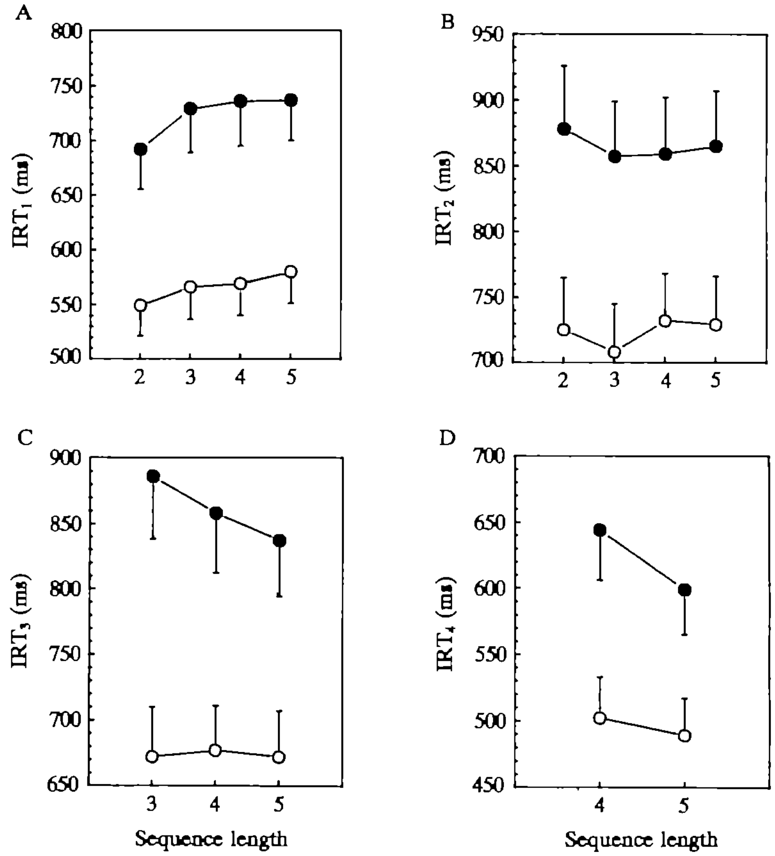

FIG. 4 Interresponse times (IRTs) for heterogeneous sequences as a function of sequence length A, B, C and D designate the mean IRTs with SE bars for IRT_1, IRT_2, IRT_3 and IRT_4, respectively Symbols as in fig. 2

lower on the MMS (mean = 27.0, SD = 1.7) than the mild PD group (mean = 28.8, SD = 1.6) (F(1,16) = 5.5, $P < 0.05$).

The results showed that while the more advanced PD group was slower in execution and made more errors (possibly due to the slowness) ($P < 0.05$), the pattern of motor programming deficits as inferred by sequence length and delay interval effects was similar for both groups. The only exception was that sequence length sometimes had less of an effect on the IRTs of repetitive movements for the mild group than the advanced group, but mild PD patients still showed similar deficits.

Correlates of sequencing performance

To explore the relationship further between sequencing and cognitive deficits in PD, performance on clinical tests of visuospatial skills (Block Design, Line Orientation), memory and attention (subtests of the Wechsler Memory Scale), mental status (MMS), and depression (Beck Depression Inventory) were correlated with sequencing performance. The pattern of delay interval and sequence length effects was examined to determine if differences between PD and controls varied as a function of performance on these tests. Using separate regression analyses with repeated measures, none of these tests explained differences between groups in delay interval effects (i.e., group×ancillary test×delay interval) for RT or IRT_1. For heterogeneous but not repetitive RT, the Block Design subtest, Line Orientation, and the Visual Reproduction subtest of the Wechsler Memory Scale significantly interacted with group and sequence length ($P < 0.05$) such that these tests were related to the pattern of sequence length effects on RT for the PD but not the control group. As all 3 tests measure some form of visuospatial processing, z scores were computed for each test and summed, producing a composite score. In a regression analysis with repeated measures, this composite score interacted with group and sequence length for heterogeneous RTs (F(3,117) = 5.45, $P < 0.01$). Follow-up analyses showed that this composite score interacted with sequence length for the PD group (F(1,22) = 8.4, $P < 0.01$) but not controls. Table 4 presents the heterogeneous RTs as a function of sequence length effects for controls and PD patients who scored in the upper and lower 33 percentile on the visuospatial composite. This table shows that PD patients who performed better on the visuospatial tasks (i.e., within the range of normals) showed as much of an increase in RT with increasing sequence length as controls whereas those who performed more poorly showed no sequence length effects. The visuospatial composite accounted for 30% of the variance in sequence length effects on heterogeneous RT ($r = 0.55$, $P < 0.025$). Thus visuospatial skills partially predicted the extent to which PD patients programmed heterogeneous sequences prior to but not during movement. This finding

TABLE 4 MEAN (SE) HETEROGENEOUS RT (ms) FOR SUBJECTS SCORING IN THE LOWER AND UPPER PERCENTILES[1] ON THE VISUOSPATIAL COMPOSITE

	Sequence length			
	2	*3*	*4*	*5*
Controls				
Lower percentile	410	450	480	480
	(43)	(48)	(51)	(54)
Upper percentile	408	420	446	452
	(20)	(24)	(25)	(30)
Parkinsonian				
Lower percentile	565	552	562	564
	(45)	(46)	(45)	(42)
Upper percentile	397	425	436	449
	(33)	(30)	(29)	(28)

[1] Visuospatial composite scores for control subjects in the lower 33 percentile ranged between −5.31 and −1.00 and for controls in the upper 33 percentile scores ranged between 1.44 and 3.10. Visuospatial composite scores for PD patients in the lower 33 percentile ranged between −8.16 and −4.70 and for patients in the upper 33 percentile ranged between −1.84 and 2.41

could not be attributed to disease severity as there was no difference between the mild and advanced PD groups on this composite measure. None of the clinical ancillary tests was related to the pattern of IRTs.

Discussion

The delay interval findings for both types of sequences showed that before movement, PD patients were able to use advance information to improve their RTs similarly to controls, but only PD patients continued to show additional benefits of a longer delay interval during the first IRT. The fact that delay interval reduced the effect of sequence length on heterogeneous RT for both groups but had no such effect on IRT_1 slope for either sequence type suggested that longer delays affected different processes before and during movement. For heterogeneous sequences, a longer delay prior to movement appears to influence programming of the entire sequence as a longer delay reduces RT slope. In contrast, for both sequence types the execution of the first response improved similarly with a longer delay, which did not affect the IRT_1 slope, suggesting that faster response times were not related to the composition of responses contained within the sequence. Instead, the effect of delay interval on IRT_1 appears specific to the first response.

Whereas before movement both groups appeared to plan repetitive movements as a single unit, once movement began the PD group performed differently and these effects could not be attributed solely to general slowness as, again, IRT slopes were not affected by delay interval. Instead, the IRT data suggest that during movement execution the PD group engages in some aspects of programming that controls have otherwise completed.

As for heterogeneous sequences, the PD group showed less programming before movement even at the longer delay interval as their RTs were less affected by sequence length, and during movement they continued to show programming deficits. Higher error rates for the first hand posture imply programming deficits for more complex sequences as no such errors were found for repetitive sequences. This may reflect a continuation of similar programming deficits seen during RT as the pattern of sequence length effects was similar for RT and IRT_1 but not for the other IRTs. Higher error rates, along with slowed IRTs for movements involving hand posture changes but not repetitions, also suggested a deficit in switching between different motor programs. Sequence length effects on IRT_3 and IRT_4 similar to those reported for IRTs of repetitive sequences, again were indicative of ongoing programming in the PD patients.

EXPERIMENT 2

One aspect of planning a sequence of movements is to resolve how many different types of responses are contained within the sequence. The results from Experiment 1 suggest this possibility as before movement the PD group appeared to plan heterogeneous but not repetitive sequences differently from controls. Experiment 2 was designed to test whether this component of planning was impaired in PD. Stimulus sequences similar to those in Experiment 1 were used, and the task was to indicate whether the sequences contained 1, 2, or 3 different hand postures. All subjects from Experiment 1 participated in this study.

Methods

The experimental task required subjects to press 1 of 3 possible keys in a row to specify the number of different types of hand postures in a sequence. Half of the subjects pressed the 3 keys from left to right with the thumb, index, and middle fingers to designate 1, 2 or 3 different hand postures; the others did the opposite.

The experimenter controlled the onset of the sequence display on the monitor. After a random delay of 250 or 2000 ms an imperative tone was presented. On completion of the response, the simulus display terminated. RT was measured from the onset of the tone to when subjects depressed a key. An error was measured when subjects made the wrong response or took longer than 2000 ms to respond, or responded before the imperative stimulus.

The stimulus displays contained sequences that were constructed similar to those in Experiment 1 except that only 3 or 5 posture sequences were used. Three different types of sequences were presented: (1) repetitive sequences of button or plate postures (1 posture); (2) heterogeneous sequences of plate and button postures (2 different postures); and (3) heterogeneous sequences of plate, button, and handlebar postures (3 different postures). Each of the 8 different stimulus sequences was presented 8 times across blocks in a random order. If subjects made an error, the trial was repeated randomly at the end of the block of trials. During practice, subjects successfully completed 2 trials of each sequence type at both delay intervals.

Results

The ANOVAs used a mixed model design with group as the between subject factor and delay interval, sequence length and sequence type (i.e., 1, 2 or 3 different postures) as the repeated factors. Of interest were the effects of group and the interactions of group with the repeated factors. Repetitive sequences of button responses were not included in the analyses so that the beginning hand posture was the same (i.e., plate) across sequence types.

Errors. There was no difference between PD and control groups in the percentage of errors before the imperative stimulus (2%) or the percentage of delayed response errors (1%). Regardless of condition, the PD group made more incorrect responses (2%) than the controls (0.4%) (F(1,41) = 8.0, $P < 0.01$).

Reaction time. The PD group did not show slower RTs than controls and there was no significant interaction of group×sequence length which indicated decision times were similar between groups regardless of sequence length. Group interacted with sequence type and delay interval (F(2,82) = 5.1, $P < 0.01$). Follow-up analyses found no differences between PD and control subjects in RTs, regardless of condition. For both groups there was a significant effect of sequence type at the 250 but not the 2000 ms delay interval such that RTs were longer for 2 and 3 posture sequences than repetitive sequences. However, Table 5 shows that the amount of improvement with a longer delay varied between groups as a function of sequence type. There was no difference between groups in the amount of improvement with a longer delay for repetitive sequences, but for 2 and 3 posture sequences, a longer delay interval improved decision times more for the PD than the control group (F(1,41) = 4.0, $P = 0.052$ and F(1,41) = 5.5, $P < 0.025$, respectively).

TABLE 5 MEAN DECISION TIMES (SE) IN SIMPLE RT (ms) AS A FUNCTION OF DELAY INTERVAL

Sequence type	*Controls*	*Parkinsonians*
Repetitive		
250	567 (52)	612 (48)
2000	383 (17)	373 (15)
Difference	184 (41)	238 (43)
Two postures		
250	651 (55)	814 (61)
2000	373 (16)	388 (20)
Difference*	278 (45)	425 (55)
Three postures		
250	690 (69)	844 (60)
2000	397 (18)	370 (18)
Difference**	293 (57)	474 (53)

* $P = 0.052$ and ** $P < 0.025$ for the difference between groups in the amount of improvement with a longer delay

Mild versus advanced PD patients

There was no difference between groups in the number of errors before the imperative stimulus, but Mann-Whitney U tests showed that the advanced PD group made more delayed response errors (mean = 3.8 vs 1.8) and more incorrect responses (mean = 3.0 vs 1.1) than the mild PD group ($P < 0.05$). Severity group interacted with delay interval (F(1,16) = 9.2, $P < 0.01$) such that patients with more advanced symptoms had longer RTs only for the 250 ms delay interval (F(1,16) = 9.1, $P < 0.01$). The advanced

PD group also showed more overall improvement (mean = 527) with a longer delay interval than the mild PD group (mean = 229) (F(1,16) = 9.2, $P < 0.01$). These findings were not influenced by sequence type or length suggesting that group differences were most likely due to the greater motor slowness of the advanced PD group.

Correlation of visuospatial skills

Of interest in this analysis was whether the pattern of delay interval and/or sequence length effects differed for PD and controls depending on their score on the visuospatial composite described in Experiment 1. A regression analysis with repeated measures showed that visuospatial skills did not explain differences between these groups in delay interval (i.e., group × composite × delay), sequence type effects (i.e., group × composite × type), or the combination of delay interval and sequence type effects (i.e., group × composite × delay × type). This suggests that making decisions about the number of different responses contained in a sequence is largely independent of visuospatial skills.

Discussion

Although RTs were not slower for PD patients, they had more difficulty determining the number of different postures in complex sequences as their decision times improved more with a longer delay only for these sequence types. This suggests that one reason underlying preprogramming problems for heterogeneous sequences (Experiment 1 RT findings) in PD may be difficulties forming an internal representation of the sequence. Specifically, problems in resolving the number of different responses could impair organizing or grouping responses according to a strategy so as to facilitate programming and subsequent decoding during execution.

GENERAL DISCUSSION

The PD patients showed different types of programming deficits depending on the complexity of sequences which was suggestive of abnormalities in several levels of motor programming. Prior to movement, RTs for sequences of repetitive hand postures did not vary with length for either group whereas for heterogeneous sequences RTs for the PD group were less influenced by sequence length. This latter finding was true even when more time was available to preprogram despite the fact that at a longer delay interval the RT slope increased less with sequence length for both groups, suggesting that all subjects had completed more programming. If the PD group was simply slower in constructing a motor program for the sequence, the RT slope might be expected to increase rather than decrease with a longer delay, similar to that of the controls at the 250 ms delay. Instead, it appears that regardless of delay interval in PD a motor program is assembled that is dissimilar to that of controls. Higher error rates in PD for the first response of heterogeneous but not repetitive sequence lengths, despite normal increases in the duration of IRT_1 with sequence length also support this conclusion. IRT_1 errors for PD patients did not decrease with a longer delay which further supports the view that this deficit was not due to slowness per se in programming sequences but rather to problems in programming more complex sequences.

Although PD patients were able to use advance information to improve their RTs, as others have shown (Stelmach *et al.*, 1987), unlike controls they continued to benefit from a longer delay during the first IRT. The percentage improvement in IRT_1 with a longer delay (9%) was considerably less than for RT (32%), but was similar for both sequence types. It may take longer for PD patients to construct a motor program for the entire sequence such that programming continues during the execution of the first response. As previously discussed, this hypothesis predicts an interaction of group ×

delay interval × sequence length for heterogeneous RT such that PD patients show more evidence of programming with a longer delay, which was not the case. Further, because the delay interval effect was similar between sequence types and did not affect the size of the sequence length effect on IRT_1, it appears that it is not the overall complexity of the sequence but, rather, the first response that benefits from additional time. This argument suggests the delay effect on IRT_1 may reflect slowed activation of the motor program for the first hand posture as EMG studies show the pattern of antagonist and agonist activity is normal but delayed in PD (Hallett *et al.*, 1977), or may reflect impaired programming of force-time parameters of movement in PD which would also produce slowing (Stelmach and Worringham, 1988). In any case, while longer delays prior to movement allowed all subjects to plan more about the sequence (i.e., reduction in RT slope with a longer delay), during the execution of the first response additional time only affected the programming of the first response.

The finding that PD patients showed faster IRTs for longer sequences (regardless of delay) suggests a separate deficit in using motor programs to control execution. Similar effects have been reported for choice RT in normal controls where the latency of the first key press became faster as the number of responses prior to an uncertain response within a sequence increased, suggesting a model where movement begins before the sequence is entirely programmed (Rosenbaum *et al.*, 1987). Extending this model to control processes during movement, the PD group had difficulty scheduling the termination of a sequence such that hand postures contained in longer sequences were more quickly executed because they had more time to identify the end of the sequence while they were executing the beginning responses. Execution of shorter sequences had to be delayed in order to plan the termination of the sequence which occurs earlier than for a longer sequence. Sequencing deficits of this type have been reported elsewhere showing that PD patients make more errors than controls in the number of repetitive finger taps in a sequence (Stelmach *et al.*, 1987).

For heterogeneous sequences, the pattern of sequence length effects differed across IRTs for both groups, suggesting that these sequences were not programmed as a single unit. While both groups show similar sequence length effects on IRT_1 and IRT_2, again PD patients made more errors executing the first hand posture. This may reflect ongoing problems (beginning during RT) assembling a motor plan for the sequence while simultaneously programming the physical parameters for the first movement. Difficulties coordinating two or more processes have been reported in PD for the performance of simultaneous movements (Benecke *et al.*, 1986) and the coordination of eye and hand movements (Warabi *et al.*, 1988).

Apart from the above programming deficits, the results also suggested that PD patients have difficulty switching between different responses, similar to others (Benecke *et al.*, 1987*a*, *b*). For heterogeneous sequences IRT_2 and IRT_3 were longer for PD than control subjects whereas for repetitive sequences no such differences were found at these response positions. PD patients also made more errors when changing hand postures but not when executing repetitions of a hand posture. This experiment did not examine whether this deficit was independent of problems programming motor parameters for individual responses. However, the fact that PD patients make more errors when initiating the first movement in a heterogeneous but not a repetitive sequence, would seem to imply that some other aspect of sequencing is also impaired.

Correlations of neuropsychological tests with sequencing performance revealed that for heterogeneous sequences only, poor visuospatial skills but not general cognitive functioning were related to deficient motor programming prior to but not during movement. This implies the visuospatial composite is more indicative of programming associated with the initial organization of responses rather than execution processes. Experiment 2 suggested that identifying the number of different hand postures contained within heterogeneous sequences may contribute to programming deficits in PD. Interestingly, poor visuospatial skills in PD were not related to slower decisions about the number of different postures, suggesting that this factor may influence motor programming independently.

Little is known about the role of visuoperceptual or visuospatial skills in motor programming, in part because of the multifaceted nature of these skills. There have been several reports of deficits in PD on a variety of visuospatial or visuoperceptual tests that minimize or eliminate motor output (Pirozzolo *et al.*, 1982; Boller *et al.*, 1984), but no study has related these deficits directly to motor programming. Studies with normal subjects have demonstrated that spatial aspects of some types of movements are represented in the motor program (Stelmach and Teulings, 1987). Stern *et al.* (1984) found that in PD poorer performance on drawing tests (which have a motor component) was related to greater difficulty performing predictable movements without visual cues. These studies, together with our findings and reports of visuospatial deficits in PD, introduce the possibility that the basal ganglia may be involved in integrating perceptual and motor systems for constructing motor programs. Still, visuomotor or visuospatial deficits have not always been observed in PD (Brown and Marsden, 1986; Taylor *et al.*, 1986).

Our results suggest that programming sequences require a variety of cognitive processes including: (1) identification of all responses; (2) ordering and grouping of the responses; (3) programming the physical parameters of responses; (4) resolving the termination of movement; and (5) controlling switching among different responses. Specifically, the absence of an RT slope for repetitive sequences, together with similar decision times in both groups for these sequences in Experiment 2, suggested that one level of programming may be identifying whether all responses are the same. PD patients seem to be able to perform this level of analysis normally. However, when all responses are not the same, Experiment 2 showed that PD patients had problems identifying the number of different responses. Based on the output from this process, responses are ordered and grouped. Repetitive sequences tend to be grouped as a single unit whereas the unit of analysis for heterogeneous sequences may be the hand posture, although structural aspects of these sequences could result in higher level grouping strategies (Povel and Collard, 1982). It is proposed that this level of programming is partially dependent on a visuospatial analysis, as only PD patients with poor visuospatial skills showed no evidence of programming heterogeneous sequences prior to movement. Another level of programming which was also impaired in PD was suggested by the IRT findings for repetitive movements and involves resolving where the sequence terminates spatially. The greater errors and slowness in PD when executing hand posture changes may also suggest abnormalities in a central executive process that controls switching between different motor programs. Finally, a level of programming related to controlling the physical parameters of individual movements was suggested by the delay effects on

IRT_1 for the PD patients only. This level may directly relate to the symptoms of bradykinesia whereas the other programming levels characterized in the present study may be more related to cognitive deficits frequently reported in PD that affect both motor and nonmotor task performance and learning, but are not closely related to bradykinesia. Thus different mechanisms may explain deficits in different levels of motor programming which raises the question, to which we now turn, of whether the basal ganglia regulate all or only some of these mechanisms.

Neuroanatomical correlates

Our results suggest that the basal ganglia play a role in several processes associated with the organization and control of hand posture sequences but two qualifications must be mentioned. The first is that the importance of different parts of the basal ganglia need to be specified as the neuroanatomical connections of the putamen and the caudate nucleus are different with the putamen projecting primarily to the supplementary motor area and the caudate projecting to the dorsolateral and lateral orbitofrontal cortex. These circuits probably serve different behavioural functions (Alexander *et al.*, 1986). Recently it has been suggested that the putamen is involved in early stages of PD and both putamen and caudate function are compromised in patients with more advanced symptoms (Nahmias *et al.*, 1985). The present study did not find qualitative differences in sequencing deficits between patients with mild and those with more advanced symptoms which may implicate the putamen and, more generally, the motor circuit in the programming and control of sequential movements. However, a study of rotary pursuit learning with the same sample of PD patients found motor learning deficits in the advanced but not mild PD group (Harrington *et al.*, 1990). One reason for this apparent discrepancy may be that motor learning requires skills more dependent on caudate function and the complex loops whereas it has been shown that the supplementary motor area is especially important for programming sequences of different finger movements (Roland *et al.*, 1980). This introduces a second limitation which is that the specific conribution of the basal ganglia to different levels of motor programming cannot be discerned without knowledge of how patients with damage to other neuroanatomical areas perform on these tasks.

A related issue concerns whether deficits in the present study apply only to motor tasks. One proposal is that the same processes which are involved in motor tasks and are performed at the level of basal ganglia can also influence nonmotor tasks (Alexander *et al.*, 1986). Planning deficits in PD have been observed on nonmotor tasks and conceptualized by some investigators as a shifting or switching disorder that did not appear to be due to frontal abnormalities in the PD patients studied (Cools *et al.*, 1984; Morris *et al.*, 1988). In the present study, there is some evidence consistent with a dysfunctional mechanism for switching or simultaneously coordinating several levels of programming such that processes normally proceeding in parallel become more serial. Some of our other findings, however, suggest the possibility of abnormalities in other mechanisms such as deficient motor program construction and a slowness programming physical parameters of responses. Future studies examining levels of programming on comparable motor and nonmotor tasks which require organizational processing may provide some insight into this issue.

ACKNOWLEDGEMENTS

The authors wish to thank Jennifer Hudson and Lee Stapp for their assistance and Dr Ellen Marder for assessing Parkinsonian status. We are also grateful to Dr Erland Nelson, Dr Douglas Barrett and Kay Chisholm for their assistance in identifying the patient group used in this study.

This research was supported by grants from the Lovelace Medical Foundation, Clinical Studies Division, Albuquerque, NM, and the Research Services of the Veterans Administration.

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(*Received July 25, 1989. Revised November 10, 1989 Accepted January 11, 1990*)

Brain (1991), **114**, 117–137

FRONTAL DYSFUNCTION AND MEMORY DEFICITS IN THE ALCOHOLIC KORSAKOFF SYNDROME AND ALZHEIMER-TYPE DEMENTIA

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SUMMARY

This study investigated the intercorrelation of 8 'frontal' tests in 32 patients with Korsakoff's syndrome and Alzheimer's disease, and examined the relationship of frontal dysfunction to impaired release from proactive interference and impoverished retrieval from retrograde memory. Amongst the frontal tests, there were statistically significant intercorrelations between 3 'fluency' tests and 3 'card-sorting' tests, although the degree of shared variance was relatively low. The relationship of another test—picture arrangement errors—was more equivocal; performance for 'cognitive estimates' was unrelated to performance in the other frontal tests, possibly because it may reflect pathology at a different frontal site. There was no evidence in this study that variability in release from proactive interference was related to measures of frontal function in either patient group, and the conditions under which these patient groups fail to show 'normal' release appear to be tightly constrained. On the other hand, the defective retrieval of retrograde memories was correlated with frontal dysfunction in both patient groups. There was a suggestion of a double dissociation with a measure of nonverbal short-term forgetting, impairment at which was related to the degree of general cortical atrophy rather than frontal dysfunction. A stepwise regression equation based on 3 frontal tests could account for 64% of the variability in retrograde memory performance within the total patient group, 68.5% in the Korsakoff group and 57% in the Alzheimer group. By comparison, the severity of anterograde memory impairment predicted only 21% of the variance in retrograde memory performance. It is concluded that frontal dysfunction produces a disorganization of retrieval processes which contributes to the temporally-extensive retrograde amnesia of these two disorders.

INTRODUCTION

Various possible functions in normal memory processes have been attributed to the frontal lobes. For example, it has been suggested that the frontal lobes are involved in the planning, organization and 'executive' aspects of memory; that they form a 'cognitive mediation' system in the encoding and retrieval of memories; and that they facilitate cognitive estimation (Luria, 1973; Shallice, 1982; Warrington and Weiskrantz, 1982; Baddeley, 1986; Duncan, 1986). Further studies suggest that the frontal lobes are involved in the semantic retrieval of words and designs, the suppression of irrelevant associations and 'release' from proactive interference, and the prevention of 'short-term' forgetting (Luria, 1976; Milner, 1982; Moscovitch, 1982; Squire, 1982; Parkin, 1987). Another group of studies implicates the frontal lobes in memory for temporal sequence and other contextual cues, in the prevention of 'spontaneous' confabulation, and in the retrieval

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of past ('remote') memories (Luria, 1976; Kapur and Coughlan, 1980; Milner, 1982; Squire, 1982; Baddeley and Wilson, 1986; Mayes, 1988).

Large frontal lesions produce deficits in these functions (e.g., Milner, 1982; Schacter, 1987; Janowsky *et al.*, 1989); and there is evidence that the memory deficits seen in Parkinson's disease, for example, may be attributable to frontal lobe dysfunction (Sagar *et al.*, 1988*a*). Frontal atrophy commonly occurs in the alcoholic Korsakoff syndrome and in Alzheimer's disease (Hubbard and Anderson, 1981; Harper *et al.*, 1987; Jacobson, 1989). Consequently certain memory deficits in these latter disorders, which are not always seen in organic amnesia, have been attributed to frontal dysfunction, such as impaired memory for the source or temporal context of information (Squire, 1982; Shimamura and Squire, 1987). However, a recent study of Korsakoff and Alzheimer patients found that memory for temporal context was unrelated to a CT scan measure of frontal atrophy, and was more closely related to the severity of anterograde memory impairment than to measures of frontal dysfunction (Kopelman, 1989). Similar findings were obtained by Pickering *et al.* (1989) in studying memory for the modality of presentation of learned material, and by M. A. Shoquerat and A. R. Mayes (unpublished results) in studying memory for spatial context. A further study reported only a very equivocal relationship between nonverbal 'short-term' forgetting and frontal dysfunction (Kopelman, 1990). Such findings suggest that these memory deficits do not necessarily arise as a consequence of frontal pathology.

The present study investigated the relationship between frontal dysfunction and two other memory deficits which have been attributed to it, namely, impaired 'release' from proactive interference and impaired retrieval from 'remote' memory. Proactive interference refers to the detrimental effect of previously learned material on the recall of newly learned information, and 'release' to the elimination of this effect when the nature of the learned material shifts in some important way, for example, in terms of semantic category. Several studies have reported that Korsakoff patients fail to show normal 'release', and it has been argued that this may result from concomitant frontal pathology (Moscovitch, 1982; Squire, 1982). Impaired retrieval of 'remote' memories in patients with Huntington's disease has been attributed to frontal dysfunction by Mayes (1986) and, in other patient groups, frontal pathology may contribute to the presence of a temporally-extensive retrograde amnesia (Mayes, 1986; Kopelman, 1987*a*; Dall'Ora *et al.*, 1989). The present study considered whether these two types of memory deficit arise as a consequence of frontal dysfunction in Korsakoff and Alzheimer patients. However, it first investigated the intercorrelations between the frontal tests employed and their relationship to other variables, such as age, intelligence, and cortical atrophy. This was because there is remarkably little information available on this, although there have been suggestions that dorsolateral and orbitofrontal functions are reflected in different tasks (Stuss and Benson, 1984; Mayes, 1988) and that patterns of impairment may differ between amnesic groups (Leng and Parkin, 1988).

SUBJECT GROUPS

Korsakoff patients

Sixteen alcoholic Korsakoff patients were tested. They were selected from a larger group of patients who had attended psychiatric hospitals around London, and were chosen (1) for conforming as closely as possible to the clinical features of the 'acute onset' subgroup identified by Cutting (1978) in a retrospective study of Maudsley Hospital patients, and (2) for being representative of patients who, on a CT scan measure, showed a greater or lesser degree of frontal atrophy (Kopelman, 1989). The patients had a similar sex ratio (11 M: 5 F) and mean age (53.7 yrs, range 38–65 yrs) to Cutting's subgroup and the Victor *et al.* (1971) sample. The rate of onset had been 'acute' in all cases for whom precise details were available. With respect to features of Wernicke's syndrome 11 out of 16 cases had a recorded history or current evidence of nystagmus and/or ophthalmoplegia, all cases had disorientation in time, 14 had ataxia, and 10 had peripheral neuropathy. All the patients had a history of very heavy and prolonged alcohol abuse (mean length of drinking history 23 (range 13–37.5) yrs). The mean time 'dry' was 5.75 (range 1–15) yrs. They were all either living in institutions or heavily dependent on institutional support. The Korsakoff patients had a mean estimated premorbid IQ of 104.3 (SD 10.6) according to Nelson's (1982) reading test, and a mean current (Wechsler) full-scale IQ of 105.25 (SD 10.7). They showed a very substantial and highly significant degree of impairment across a range of anterograde memory tests, including Logical Memory immediate and delayed (Wechsler, 1945), Object Learning (Kendrick, 1985) and the Recognition Memory Test (Warrington, 1984) (*see* Table 1).

Alzheimer patients

Sixteen patients with a dementing disease of Alzheimer type were tested. Diagnosis was made according to clinical criteria (history, examination, and physical investigations), implicit in which is the exclusion of other possible causes of dementia. All patients had a history of progressive cognitive impairment. The mean duration of symptoms was 2.9 yrs (range 9 mos–6 yrs). None of the patients was hypertensive, and none had a history of cerebrovascular disease. For inclusion in the study, patients were required to have psychometric evidence of a generalized impairment of cognitive function and CT scan evidence of cortical atrophy. The sex ratio was 5 males: 11 females, and mean age 68.7 (range 58–76) yrs, reflecting the different sex and age distributions of this disorder from Korsakoff's syndrome. Memory complaints were prominent in all patients, 14 showed impaired copying and drawing (*cf* Moore and Wyke, 1984), and the remaining 2 showed other evidence of visuospatial dysfunction. However, inclusion in the study required that subjects should be able to perceive newspaper photographs without difficulty. Mean estimated premorbid IQ on the reading test was 106.1 (SD 8.2) and mean current IQ was 88.6 (SD 11.1), a mean difference of 17.5 points. On the anterograde memory tests mentioned above, the Alzheimer group obtained mean raw scores closely similar to those of the Korsakoff group (Table 1).

TABLE 1. ANTEROGRADE MEMORY TEST SCORES AND CT SCAN DATA*

	Healthy controls (n = 16)	*Korsakoff patients* (n = 16)	*Alzheimer patients* (n = 16)
Logical Memory			
immediate	12.1 (±4.2)	4.4 (±1.6)	3.5 (±1.9)
delayed (45 min)	10.5 (±3.9)	0.5 (±0.9)	0.7 (±1.1)
Kendrick Object Learning	42.25 (±8.0)	16.3 (±4.2)	13.9 (±7.3)
Warrington Recognition Memory			
words	46.75 (±2.7)	29.2 (±6.75)	28.4 (±5.7)
faces	39.6 (±4.6)	28.9 (±5.5)	30.1 (±3.4)
CT scan measures			
VBR	Not available	12.4 (±2.8)	16.3 (±3.75)
'cortical atrophy' scale	Not available	1.4 (±0.9)	2.6 (±1.15)

* Means ± SD

Control subjects

Sixteen control subjects were tested, who overlapped with the two patient groups in terms of sex and age distributions (7 male: 9 female, mean age 61.75, range 40–78 yrs); and the control group could be subdivided into (age and sex-matched) Korsakoff and Alzheimer comparison groups. The control subjects consisted of healthy elderly subjects living in sheltered accommodation in south London, the healthy relatives of some of the patients, and nonacademic staff from the Institute of Psychiatry. Mean estimated premorbid IQ was 108.2 (SD 11.2) and current IQ = 111.6 (SD 9.8). Mean scores on the anterograde memory tests were close to the published age-related population norms for British and Canadian populations (Table 1).

CT SCAN ASSESSMENT

Planimetric measurements of ventricular-brain ratio (VBR) and standardized ratings of sulcal and sylvian fissure widening (on a 0–5 'cortical atrophy' scale) were available for the two patient groups (*see* Baldy *et al.*, 1986). Table 1 shows that the Alzheimer group had a significantly greater degree of cerebral atrophy than the Korsakoff group on these two measures ($t = 3.37$, $P < 0.01$ and $t = 3.45$, $P < 0.01$, respectively).

EXPERIMENT 1 FRONTAL TEST PERFORMANCE

In this experiment, the performance of the patient groups on 8 measures of frontal function was investigated with particular reference to the intercorrelation of the tests employed and their relationship to the age of the subjects and measures of intelligence and cortical atrophy.

Methods

FAS Verbal Fluency. Subjects were required to produce as many words as they could beginning with the letters F, A, or S in the course of 1 min each. Varying forms of the same word (e.g., sit, sitting) and the names of people or places were not acceptable (Benton, 1968; Miller, 1984).

FAS with Miller's Correction. This was as above, but Miller's (1984) formula correcting for verbal IQ was applied. This is based on the scaled scores obtained on 3 verbal IQ subtests (vocabulary, comprehension, similarities). A positive score indicates a degree of frontal dysfunction disproportionate to any impairment in verbal IQ, whereas a negative score indicates frontal performance superior to what would be expected on the basis of verbal IQ.

Alternating birds and colours. Subjects were required to give the names of birds and colours alternately, naming as many as possible in 1 min. Repetitions were not permitted. This test was described by Newcombe (1969) and, more recently, Lishman *et al.* (1987) have reported that impairment in alcoholics showed a significant correlation with changes in frontal tissue density on CT scan.

Modified Weigl Test. Three sets of 12 cards each were employed with designs varying in terms of either colour and form, colour and number, or form and number. A set of cards was placed on a table in a random fashion, and the subject was required to sort each set according to one 'rule' (e.g., colour) and then another (e.g., form) (*cf* Weigl, 1941). Maximum score for this test = 6.

Card-sorting categories. Nelson's (1976) version of the Wisconsin Card-sorting Test was employed. This required subjects to learn a rule by which cards, whose patterns varied in terms of colour, form, and number, should be sorted. There were 48 cards in the test; the experimenter gave the subject feedback whether each response was correct or not; and the rule changed after 6 successive correct responses. The number of category rules 'learned' (i.e., 6 successive correct responses) was scored.

Card-sorting perseverations. This was as above, but the absolute number of perseveration errors, according to Nelson's (1976) definition, and the proportion of perseverative relative to total errors, were recorded.

Cognitive estimates. Subjects were required to give estimates of the dimensions, age, number, and speed of 10 items, e.g., the height of the Post Office (Telecom) tower. All 10 items were taken from Shallice and Evans's (1978) paper, and scoring was according to standardized criteria in terms of the size of the subjects' errors.

Picture arrangement errors. This consisted of the Picture Arrangement subtest of the Wechsler Adult Intelligence Scale, scored in terms of the number of pairs of pictures left erroneously in the presented order. This method of scoring was recommended as a measure of frontal dysfunction by McFie and Thompson (1972), and has been employed more recently by Sagar *et al.* (1988*a*).

Statistical analysis

A large number of correlations was performed, giving rise to the risk that some might appear statistically significant by chance. However, here and elsewhere in the paper, it was the overall pattern of significance values which was of greatest importance and which will be considered in the Discussion.

Results

Fig. 1 plots the mean scores of the groups' performance across the 'frontal' tests. Using a one-way analysis of variance, all the group differences were statistically significant at $P < 0.001$, except for the Miller correction score which was significant at $P < 0.005$. On the Miller test, a score of zero meant that any impairment on FAS verbal fluency was proportionate to any decrement on the verbal intelligence subtests, whereas a positive score indicated a disproportionate degree of 'frontal' dysfunction. The histogram shows that the Alzheimer group did not differ significantly from the controls on this test, whereas the Korsakoff group showed a significantly raised score ($t = 3.87$, $P < 0.001$), indicating that the Alzheimer group manifested a consistent degree and the Korsakoff group a disproportionate degree of 'frontal' dysfunction relative to verbal IQ. On all the other tests, the Alzheimer group was significantly impaired relative to the healthy controls. The Korsakoff group's performance was intermediate between that of the healthy controls and the Alzheimer group on all these other tests except cognitive estimates, at which it closely matched that of the Alzheimer group.

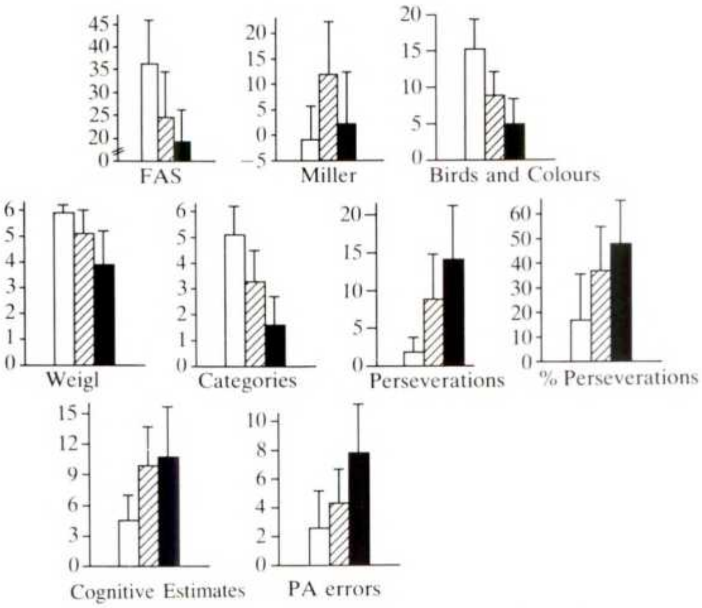

FIG. 1. Mean performance by healthy controls, Korsakoff patients and Alzheimer patients on frontal tasks. Each bar = 1 SD. Categories and perseverations refer to performance on the card-sorting task, perseverations to the absolute number of perseverations, and % perseverations to the proportion of total errors they accounted for. PA errors = picture arrangement errors. Open areas = healthy controls; hatched areas = Korsakoff patients; filled areas = Alzheimer patients.

Table 2 shows the relationship between performance on these tests and age, estimated premorbid and current intelligence, and the CT scan measures of ventricular-brain ratio (VBR) and cortical atrophy. The correlations reported are those obtained in the total (Korsakoff and Alzheimer) patient group (n = 32) Performance on the frontal tests correlated most consistently with current IQ; all these correlations were statistically significant and in the expected direction. However, there were also a number of statistically significant correlations between frontal test performance and age, estimated premorbid IQ, and the 2 CT scan measures. The 2 CT scan measures were correlated most strongly with the degree of general cognitive decline, as estimated by the NART–IQ difference ($r = 0.51$, $P < 0.01$ and $r = 0.61$, $P < 0.001$, respectively).

TABLE 2 CORRELATIONS OF FRONTAL TEST PERFORMANCE WITH AGE, INTELLIGENCE, AND CORTICAL ATROPHY WITHIN THE TOTAL PATIENT GROUP (n = 32)

	Age	*Premorbid IQ (NART)*	*Current IQ (full scale)*	*NART-IQ difference*	*CT. VBR*	*CT cortical atrophy*
FAS Verbal Fluency	−0.27	0.44*	0.43*	−0.14	−0.06	−0.16
Miller correction	−0.23	0.13	0.55***	−0.51**	−0.18	−0.16
Alternative birds/colours	−0.43**	0.10	0.40*	−0.37*	−0.41**	−0.35*
Modified Weigl Test	−0.38*	−0.02	0.48**	−0.54***	−0.12	−0.34*
Card sorting: categories	−0.58*	0.19	0.53***	−0.44**	−0.45**	−0.34*
Card sorting percentage perseverations	0.30*	−0.44**	−0.35*	0.05	−0.06	0.05
Cognitive Estimates	−0.08	−0.40*	−0.53***	0.28	0.36*	0.17
Picture Arrangement errors	0.32*	−0.09	−0.49**	0.47**	0.32*	0.34*
CT scan VBR	0.27	0.19	−0.33*	0.51**	–	0.43**
CT cortical atrophy	0.38*	0.28	−0.37*	0.61***	0.43**	–

* $P < 0.05$, 1-tailed; ** $P < 0.01$, 1-tailed, *** $P < 0.001$, 1-tailed.

Table 3 shows the intercorrelations of frontal test performance in the total patient group, Korsakoff patients, and Alzheimer patients. In view of the significant correlations between current IQ and each of these tests, the effect of IQ has been partialled out in these matrices. It can be seen that, within the total patient group, 10 out of 15 of the correlations involving the fluency tests (FAS, Miller correction, birds and colours) and the card-sorting tests (Weigl, categories, perseverations) were statistically significant, although the degree of shared variance tended to be rather low. In general, this pattern also held good within the Korsakoff group taken in isolation, whereas in the Alzheimer group the intercorrelations were statistically significant only for the three fluency tests. Picture arrangement errors produced a somewhat different pattern of results, correlating significantly with performance on the fluency tests in the Alzheimer group but not in the Korsakoff group. On the other hand, cognitive estimates' scores did not correlate significantly with performance on any of the other frontal tests within the total patient group and, in the Korsakoff group, a correlation of 0.52 with the modified Weigl score was in the opposite direction from prediction. However, performance at cognitive estimates did correlate significantly with various other variables, namely premorbid and current IQ and with CT scan VBR (Table 2), and with indexes of the severity of retrograde and anterograde amnesia (Experiment 3 below).

Although there were differences between the Korsakoff and Alzheimer groups in terms of the size or direction of these correlations, only in one instance (picture arrangement errors × birds and colours) out of 28 comparisons did the difference reach statistical significance at a $P < 0.05$ level, and this may have occurred by chance.

Discussion

These findings confirm that Alzheimer patients are severely impaired, and Korsakoff patients more moderately impaired, at tests of 'frontal' function. The differences between the two groups cannot simply reflect a unitary 'severity' factor in brain pathology, because the groups were matched in terms of mean

TABLE 3 INTERCORRELATIONS BETWEEN 'FRONTAL' TESTS AFTER PARTIALLING FOR CURRENT IQ

	FAS Verbal Fluency	*Miller Correction*	*Birds and Colours*	*Modified Weigl*	*Card-sorting categories*	*Card-sorting % perseverations*	*Cognitive Estimates*
Total patients (n = 32)							
FAS Verbal Fluency							
Miller Correction	−0.82***						
Birds and Colours	0.45**	−0.38*					
Modified Weigl	0.26	−0.33**	0.53**				
Card-sorting categories	0.37*	−0.19	0.53**	0.35*			
Card-sorting % perseverations	−0.41**	0.23	−0.40*	−0.06	−0.24		
Cognitive Estimates	0.05	−0.08	−0.08	0.25	−0.10	0.04	
Picture Arrangement Errors	−0.28	0.35*	−0.27	−0.20	0.07	0.09	−0.07
Korsakoff patients (n = 16)							
FAS Verbal Fluency							
Miller Correction	−0.89***						
Birds and Colours	0.54*	−0.41					
Modified Weigl	0.07	−0.10	0.51*				
Card-sorting categories	0.63**	−0.56*	0.64**	0.22			
Card-sorting % perseverations	−0.46*	0.29	−0.47*	−0.10	−0.63**		
Cognitive Estimates	−0.20	0.22	0.02	0.52	−0.27	0.10	
Picture Arrangement Errors	−0.07	0.12	0.39	0.33	0.28	−0.09	−0.01
Alzheimer patients (n = 16)							
FAS Verbal Fluency							
Miller Correction	−0.77***						
Birds and Colours	0.46*	−0.55*					
Modified Weigl	0.43	−0.61**	0.60**				
Card-sorting categories	0.12	0.06	0.34	0.27			
Card-sorting % perseverations	−0.40	0.25	−0.28	−0.07	−0.20		
Cognitive Estimates	0.25	−0.40	−0.41	−0.03	−0.34	0.09	
Picture Arrangement Errors	−0.48*	0.65**	−0.52*	−0.41	0.22	0.14	0.07

* $P < 0.05$, 1-tailed, ** $P < 0.01$, 1-tailed, *** $P < 0.001$, 1-tailed

raw scores on anterograde memory tests. Nor can it simply reflect a deterioration in general cognitive function, because patients in the Korsakoff group were intact (and those in the Alzheimer group impaired) in terms of full-scale IQ.

With regard to the severity of impairment at particular tests, the Alzheimer patients produced a mean score for FAS verbal fluency (19.25 ± 7.0) closely similar to that obtained by Miller (1984) in patients with localized frontal lesions (20.6), and a mean score for card-sorting (%) perseverations (47.9 ± 17.3) similar to that obtained by Nelson (1976) in patients with frontal lesions (42 ± 24). In both of these instances, the Korsakoff means indicated a lesser degree of impairment (means = 24.5 ± 10.0 and 36.9 ± 18.0, respectively). When Miller's (1984) formula was applied, the Alzheimer patients' mean FAS score was found to be compatible with their verbal IQ, whereas the Korsakoff group showed a disproportionate degree of frontal dysfunction, consistent with neuroradiological and neuropathological findings of frontal atrophy in this group (Harper *et al.*, 1987; Jacobson, 1989). For alternating birds and colours, and Picture Arrangement errors, the Alzheimer and the Korsakoff groups, who both showed *bilateral* frontal atrophy, performed worse than the patients with unilateral frontal lesions reported by Newcombe (1969) and McFie and Thompson (1972), respectively. On cognitive estimates, both groups (means = 10.7 ± 5.0 and 9.9 ± 3.8) performed similarly to a group of postencephalitic patients (mean = 11.6 ± 3.8) reported by Leng and Parkin (1988), but worse than those authors' 7 Korsakoff patients (mean = 4.0 ± 1.3) for reasons which are not entirely clear.

One possible interpretation of the correlation between full-scale IQ scores and frontal test performance is that, because a number of IQ subtests (e.g., similarities, picture arrangement) tap frontal lobe functions, it is unnecessary to partial out the effect of IQ in evaluating the intercorrelations between frontal tests. The alternative interpretation is that 'frontal tests' tap other 'nonfrontal' cognitive functions, in which case it is necessary to partial out the effect of IQ. In the present study, the latter more conservative strategy has been adopted. The intercorrelations between frontal tests tended to be higher before partialling for IQ and, for example, within the total patient sample, FAS verbal fluency correlated significantly with all the other frontal tests except cognitive estimates (Kopelman, 1989, Table 6).

To a substantial extent, the intercorrelations between the frontal tests obtained in the present study are reassuring, even if the degree of shared variance tended to be rather low. For example, significant correlations were obtained between FAS verbal fluency and card-sorting categories and perseverations in both the Korsakoff and total patient samples, whereas Pickering *et al.* (1989) failed to obtain a significant correlation between FAS and perseverations in a sample of 7 amnesic patients (5 of whom were Korsakoff alcoholics). In the Alzheimer group, the correlation between FAS verbal fluency and perseverations only just failed to reach statistical significance, and the other fluency by card-sorting correlations may have been attenuated by scores which were close to 'floor'.

The two tests which correlated least well with the others were picture arrangement errors and cognitive estimates. Neither has been as frequently used, nor as thoroughly validated, as a measure of frontal lobe dysfunction as the other, more conventional tests. In the Alzheimer group, but not the Korsakoff group, picture arrangement errors correlated significantly with fluency scores, and the correlation with modified Weigl score only just failed to reach statistical significance. The Korsakoff group performed relatively well at picture arrangement, consistent with their intact IQ, and possibly that contributed to the failure of picture arrangement errors to correlate significantly with the other frontal tests in this group. Cognitive estimates showed no significant association with any of the other frontal tests in either patient group. The significant correlations between this test and measures of IQ and retrograde and anterograde amnesia indicate that it may be better conceptualized as measuring some aspect of access to semantic memory. Alternatively, it may measure a different locus of frontal lobe pathology from the other tests, as Leng and Parkin (1988) have suggested, but the failure to replicate these authors' Korsakoff findings means that this claim requires more thorough substantiation.

These results have implications for the interpretation of Experiments 2 and 3 below. The finding of significant correlations between the 'frontal' tests, even after partialling for IQ as an index of general cognitive decline, provides 'construct validity' (Cronbach and Meehl, 1955) for the belief that the tests are measuring some more specific underlying factor. Studies of patients with focal brain lesions indicate that frontal pathology is most likely to be the basis of this common factor (e.g., Milner, 1982; Miller, 1984; Janowsky *et al.*, 1989), and we know from CT and neuropathological evidence that both patient groups exhibit frontal atrophy (Hubbard and Anderson, 1981; Harper *et al.*, 1987; Lishman *et al.*, 1987; Shimamura *et al.*, 1988). It might be argued that the cerebral atrophy in alcoholics can be (at least partially) reversible (Ron *et al.*, 1982), but this seems unlikely to be pertinent in the present Korsakoff group, who had been 'dry' for a mean of 5.75 yrs (range 1 – 15 yrs). Moreover, frontal neuronal loss can be detected in Korsakoff patients at postmortem (Harper *et al.*, 1987). In short, it seems reasonable to infer that where a consistent pattern of correlations emerges between performance at these tasks and some other variable, this is likely to reflect some aspect of frontal function. On the other hand, the low degree of shared variance between the tests cautions us that any single measure taken in isolation may prove an unreliable index of 'frontal' performance.

EXPERIMENT 2. RELEASE FROM PROACTIVE INTERFERENCE

The second experiment investigated release from proactive interference (PI). Only one study to date (Cushman *et al.*, 1988) has investigated this phenomenon in Alzheimer patients, and that study may have been confounded by a 'floor' effect. In Korsakoff patients, failure to 'release' from PI has commonly been observed (Butters and Cermak, 1980; Warrington, 1982), although this is not seen under all experimental conditions (Winocur *et al.*, 1981, Freedman and Cermak, 1986). It has been argued that this failure to show normal 'release' may be a consequence of concomitant frontal pathology because, whereas some amnesic patients show normal 'release', patients with frontal lobe lesions do not (Moscovitch, 1982, Squire, 1982). In the present study, the Korsakoff patients had been selected for showing a greater or lesser degree of frontal atrophy on CT scan (for details, *see* Kopelman, 1989), and the aim was to investigate the relationship of impaired 'release' to performance on the various tests of frontal function within the two patient groups.

Methods

The procedure was a modification of that used by Craik and Birtwistle (1971), Winocur *et al.* (1981) and Moscovitch (1982). Each subject was tested on four 10-word lists in each of two 'blocks' of trials. The words were read by the experimenter at a rate of 1 word per 3 s, and the subject was required to recall as many words as possible immediately after completion of the list. No limit was placed on the amount of time required during recall, and the next list was not begun until it was evident that no more words could be remembered. In one block of trials, all four lists were words drawn from the same taxonomic/semantic category ('no shift' condition). In the other block of trials, the first three lists were all from a certain taxonomic category, but a different category was used for the fourth list ('shift condition'). The order of these experimental conditions was counterbalanced across subjects. The word lists consisted of animal names, names of professions, and vegetable names. The words were taken from Bousfield's studies of associative clustering, and the lists were matched as closely as possible in terms of the mean frequency of words from each category (Bousfield, 1953; Bousfield and Cohen, 1955; Bousfield *et al.*, 1958; Loess, 1967).

Results

Fig. 2 shows the pattern of results at this test. The discontinuous curves indicate findings in the 'no shift' condition, and the continuous curves indicate performance in the 'shift' condition. As expected, the overall performance of the controls was superior to that of the two patient groups; there was a highly significant main effect of groups for performance during the first 3 trials ($F(2,45) = 26.8$, $P < 0.001$). Although there was an overall tendency for the controls to show a greater decline in performance over the first 3 trials, the groups did not differ significantly within the 'shift' condition in terms of the decrement in their performance between trials 1 and 3 ($F(2,45 = 2.95$, n.s.), i.e., in the 'build-up' of PI within this condition.

Fig. 2 indicates that all the groups showed release from PI in terms of improved performance between trials 3 and 4 in the 'shift' condition. Statistical analysis of performance in these two 2 trials showed that there was a main effect of group ($F(2,45) = 18.6$, $P < 0.001$) as well as the main effect of (shift/no shift) condition ($F(1,45) = 9.8$, $P < 0.005$) and a significant condition×trial interaction effect ($F(1,45) = 22.75$, $P < 0.001$). However, there were no significant group×condition or group×condition×trial interactions, i.e. the groups did not differ in terms of release from PI.

In terms of the association between 'release' and frontal function, correlations within the total patient group were low and often in the opposite direction from that predicted. Using two-tailed tests, there were

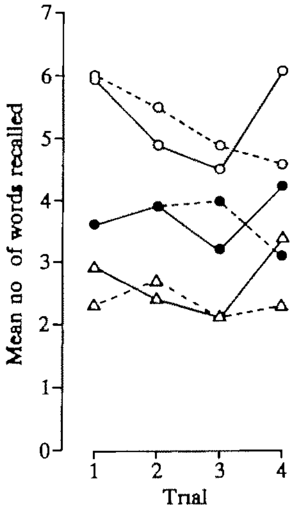

FIG. 2 Release from proactive interference. Open circles = healthy controls, filled circles = Korsakoff patients; triangles = Alzheimer patients.

no significant correlations between scores on any of the frontal tests and 'difference' scores between trials 3 and 4 in the 'shift' condition (range = −0.19 to 0.32). Subtracting 'difference' scores in the 'no shift' condition from those in the 'shift' condition, and the use of ranking procedures, also failed to give any significant correlations with the result of frontal tests. Nor did a subgroup of 8 Korsakoff patients, who had a greater degree of frontal atrophy on CT scan (Kopelman, 1989), differ significantly in their performance on this test from 8 Korsakoff patients who showed only a very minimal degree of frontal atrophy on their CT scans.

Discussion

Although unexpected, the present finding of normal 'release' from PI in the two patient groups was not entirely unprecedented. In other amnesic groups, normal 'release' has previously been reported. For example, Moscovitch (1982) reported normal 'release' in patients with temporal lobe excisions, Cermak (1976) in a post-herpes encephalitic patient, and Squire (1982) in a patient amnesic from a penetrating brain lesion and in depressed patients following ECT. Moreover, Winocur *et al.* (1981) reported that Korsakoff patients showed normal 'release' if they were told in advance to expect the shift in taxonomic category or if a previous shift had occurred. Freedman and Cermak (1986) replicated the latter result, and these authors' data showed a trend towards normal release in a group of Korsakoff patients on the occasion of the first taxonomic shift. It should also be noted that some studies appear to have selected patients for inclusion who had shown a 'build-up' of PI in the initial few lists (*see* Moscovitch, 1982), whereas the present subjects were not selected for this. Moreover, the difference between 'release' and 'no release' in such experiments often amounts to no more than the recall of 1 extra word per list.

One possible explanation of differences between groups or between studies is that impaired 'release' reflects the severity of memory impairment in amnesic patients. However, Moscovitch (1982) pointed out that his patients with temporal lobe excisions (who showed 'release') were more severely impaired at verbal memory than were comparison groups of 'frontal' and Korsakoff patients (who did not show 'release'). Moreover, in the present study, there was no significant correlation between an index of the severity of anterograde memory impairment and the presence or absence of 'release'.

A second possible explanation is that impaired 'release' is a sign of frontal dysfunction (*cf.* Moscovitch, 1982). Squire (1982) obtained a significant (rank) correlation with an index of frontal dysfunction, although only after pooling the results of a 'release' experiment with those of a 'temporal context' experiment. However, within the present study, the presence or absence of 'release' was not correlated with performance on any of the frontal tests employed. Consistent with this, Freedman and Cermak (1986) and Janowsky *et al.* (1989) have obtained normal release from PI in patients with frontal lesions who lacked any memory impairment, and Sagar *et al.* (1989) in parkinsonian, patients who were impaired on a 'frontal' task, whereas Becker *et al.* (1990) failed to obtain release in a Korsakoff patient who showed normal performance on frontal tasks.

In the light of these apparently conflicting findings, Freedman and Cermak (1986) and Janowsky *et al.* (1989) have argued that suppression of PI release may be a consequence of *combined* memory and frontal dysfunction, but the present study would not appear to support even that conclusion. However, a difference between the present study and many others was that only 3 learning trials were employed before the taxonomic 'shift', and this may have been insufficient for enough PI to accumulate to suppress 'release'. Comparing trials 1 and 3, the controls showed a trend towards greater decline in performance (paired t = 4.99, $P < 0.001$) than did the Korsakoff group (t = 1.96, $P < 0.05$, 1-tailed) or the Alzheimer group (t = 2.28, $P < 0.05$). If this were indeed the explanation of preserved PI release in the present study, it suggests that suppression of 'release' in Korsakoff patients operates only within very tight constraints. Taken together with the Winocur *et al.* (1981) and Freedman and Cermak (1986) results, it appears that Korsakoff patients will show impaired 'release' only after 4 or more word-list trials, on the first occasion of testing only, and when the patients have not been given any warning of the category shifts; and the same may well be true of early Alzheimer patients. The determinants and constraints of this phenomenon are currently being investigated in further studies, but the present findings indicate that impaired release from PI is not a very resilient phenomenon, and they do not support the view that it is a clinically reliable measure of frontal dysfunction.

EXPERIMENT 3. RETROGRADE AMNESIA

The third experiment investigated the relationship of frontal function to retrieval from remote memory. Various authors have suggested that 'cortical' (Shimamura and Squire, 1986) or 'frontal' factors (Mayes, 1986; Kopelman, 1987*a*) may contribute to the recall of remote memories. Others have suggested that the frontal cortex plays a supervisory, coordinating role in memory (Shallice, 1982; Warrington and Weiskrantz, 1982) which, when it goes out of control, can result in spontaneous or 'fantastic' confabulation (e g., Kapur and Coughlan, 1980; Baddeley and Wilson, 1986; Kopelman, 1987*b*). A recent study failed to find any correlation between a CT scan measure of the degree of frontal *atrophy* in Korsakoff patients and performance at several tests of remote and autobiographical memory, except for a trend for Korsakoff patients without frontal atrophy to show a better response to the retrieval cues provided by recognition testing than those with frontal atrophy (Kopelman, 1989). A similar study found that impaired autobiographical memory was associated with 'widespread' brain damage, but failed to find any specific association with either frontal atrophy or a (single) test of frontal dysfunction (Dall'Ora *et al.*, 1989). The present investigation extends the findings of the Kopelman (1989) study by providing a detailed analysis of the correlations between frontal *function* and remote memory tests.

The study will investigate the patterns of correlations between frontal lobe tests and a retrograde memory quotient, an anterograde memory quotient, and a retrograde/anterograde difference score, as a means of identifying any specific association between frontal dysfunction and retrograde loss. In addition, it will examine the separate correlations with 'early' and 'more recent' items within the remote memory tests.

Methods

The tests employed will be outlined only in brief, as they have previously been described in detail elsewhere (Kopelman, 1989).

News events test – recall. Subjects were shown 50 pictures of famous news events, which occurred between 1935 and 1984, and were asked a 'recall' question about the event depicted.

New events test – recognition. Following the recall question, subjects were given a 5-choice recognition question, whether or not they had recalled correctly.

Famous personalities test (Stevens, 1979). Subjects were asked to identify whether they recognized as familiar or not 160 names, 60 of whom were fictitious, 20 'very famous', and 80 of whom were people prominent in 1 of 4 time-periods from the 1930s onwards.

Personal semantic memory schedule (Kopelman *et al.*, 1989). This is a semi-structured interview probing subjects' memories for *facts* about their own past lives, covering 'background' information and memories from 3 different time-periods (childhood, early adult life, and recent facts). The authenticity of retrieved memories was checked against a number of different sources (medical records, next-of-kin, current care staff).

Autobiographical incidents schedule (Kopelman *et al.*, 1989). Subjects were required to retrieve and describe *events* or incidents from their past lives in response to specific cue words, relating to the same 3 time-periods. Authenticity was again checked against a number of sources.

Retrograde memory quotient (RMQ). Each subject's score on each test was assigned an index score in terms of the mean and SD obtained in the healthy control group with the controls' mean = 100, and 1 SD = 15 points. The mean of the 5 index scores for each subject was taken as the subject's RMQ.

Anterograde memory quotient (AMQ). On the basis of the Wechsler logical memory immediate recall score, Kendrick object learning, and Warrington word and face recognition scores, this quotient was calculated in the same way as RMQ, except that index scores were determined on the basis of published norms rather than on scores in the control group (*see* Kopelman, 1989).

R/A difference score (= RMQ − AMQ). A large discrepancy indicated that retrograde memory was relatively spared, whereas a small discrepancy implied similarly severe impairment in both retrograde and anterograde memory. The former would be consistent with a temporally-limited and the latter with a temporally-extensive retrograde loss.

Results

Table 4 shows the mean RMQ, AMQ, and R/A difference scores in each group. A number of preliminary points should be made. First, RMQ was calculated on the basis of means and standard deviations in the control group (hence control mean = 100.0), whereas AMQ was based on population norms (hence control mean = 103.2). Secondly, the index score for each subject for each test was determined on the basis of 1 SD = 15 points; but the SDs for RMQ and AMQ (the means of the index scores) generally worked out as approximately 10 points. Thirdly, the Korsakoff patients' raw scores on the anterograde tests closely matched those of the Alzheimer patients, but their mean AMQ was somewhat lower because of the age correction built into the published norms.

TABLE 4 MEAN RMQ, AMQ AND R/A DIFFERENCE SCORES (±1 SD) IN HEALTHY CONTROLS, KORSAKOFF AND ALZHEIMER PATIENTS

	RMQ	*AMQ*	*R/A difference scores*
Healthy controls	100.0(±10.15)	103.2(±11.5)	−3.2(±12.4)
Korsakoff patients	70.3(±11.6)	54.7(±10.6)	15.6(±10.3)
Alzheimer patients	62.1(±15.7)	60.2(±9.5)	1.9(±12.3)

The R/A difference score was minimal in both the control and the Alzheimer groups, indicating that the latter group showed a consistent degree of impairment across the retrograde and the anterograde tests. On the other hand, the Korsakoff group obtained a mean R/A difference score of 15.6 points, indicating relative sparing of retrograde memories and consistent with the significantly steeper temporal gradient (i.e., relative sparing of the most distant memories) found in this group in comparison with Alzheimer patients (Kopelman, 1989). In terms of R/A difference scores, there was a significant effect of groups ($F(2,45) = 11.2$, $P < 0.001$), the Alzheimer patients differing significantly from the Korsakoff group ($F(1,30) = 11.9$, $P < 0.005$) but not from the controls ($F(1,30) = 1.35$, n.s.).

Table 5 shows the correlations between the frontal test scores and RMQ, AMQ and R/A difference scores in the total patient group, Korsakoff group, and Alzheimer group, respectively. In the total patient group, 6 out of 8 of the frontal tests correlated significantly with RMQ and 5 out of 8 with R/A difference scores, whereas only 1 frontal test (cognitive estimates) correlated significantly with AMQ. The figures in parentheses indicate that the same trends were apparent in the correlations after partialling for IQ. However, inspection of the correlations for the separate Korsakoff and Alzheimer groups indicates that this overall pattern was largely attributable to the performance of the Korsakoff group. In terms of the first 6 frontal tests, those which tend to intercorrelate significantly together (Experiment 1), 3 of the tests correlated significantly with RMQ and 3 with R/A difference scores in the Korsakoff group, but none of the 6 correlated significantly with AMQ. In the Alzheimer group, on the other hand, 2 of these 6 tests correlated significantly with RMQ, 3 with AMQ, and none with R/A difference score. In terms of the mean correlations between these 6 frontal tests and the memory quotients, there was a significantly higher correlation with RMQ than AMQ in the Korsakoff group (mean $r(RMQ) = 0.36$, mean $r(AMQ) = 0.08$, paired $t = 4.12$, $P < 0.01$) and no significant difference in the Alzheimer group (mean $r(RMQ) = 0.34$, mean $r(AMQ) = 0.45$, paired $t = 2.09$, n.s.). In short, impaired frontal function was correlated with retrograde but not anterograde memory loss in the Korsakoff group, and with both retrograde and anterograde loss in the Alzheimer group.

A further analysis was conducted to see whether frontal dysfunction was particularly associated with the loss of 'early' or 'more recent' memories on the remote memory tests. On the news events tests, 'early' memories referred to items from 1935 to 1964; on the 'personalities' test to items from the first 3 'time-blocks' according to the standardized study (Stevens, 1979); and on the personal semantic and autobiographical schedules to facts and events from 'childhood' and 'young adult' life. 'More recent' memories referred to items from 1965 to 1984 on the news events tests; to items from the most recent 'time-block' on the

TABLE 5 CORRELATIONS BETWEEN FRONTAL TESTS AND RMQ, AMQ, AND R/A DIFFERENCE SCORES

	RMQ		*AMQ*		*R/A difference*	
Total patients (n = 32)						
FAS Verbal Fluency	0.52**	(0.42*)	0.27	(0.22)	0.36*	(0.23)
Miller Correction	0.10	(−0.22)	−0.24	(−0.39*)	0.29	(0.10)
Birds and Colours	0.59***	(0.50**)	0.26	(0.22)	0.43**	(0.33*)
Modified Weigl	0.33*	(0.14)	0.08	(0.01)	0.29	(0.13)
Card-sorting categories	0.51**	(0.35*)	−0.00	(−0.10)	0.56***	(0.45**)
Card-sorting % perseverations	−0.40*	(0.29)	−0.02	(0.03)	−0.42**	(−0.32*)
Cognitive Estimates	−0.64***	(0.52**)	−0.48**	(−0.47**)	−0.32*	(−0.14)
Picture Arrangement errors	−0.21	(0.03)	−0.22	(−0.17)	−0.06	(0.17)
Korsakoff patients (n = 16)						
FAS Verbal Fluency	0.66**	(0.62**)	0.33	(0.22)	0.41*	(0.40)
Miller Correction	−0.09	(0.39)	−0.05	(−0.33)	−0.05	(−0.09)
Birds and Colours	0.49*	(0.44*)	0.20	(0.09)	0.35	(0.34)
Modified Weigl	0.03	(0.04)	−0.09	(−0.09)	0.12	(0.12)
Card-sorting categories	0.34	(0.38)	−0.02	(−0.04)	0.41*	(0.41)
Card-sorting % perseverations	−0.46*	(−0.35)	0.01	(0.22)	−0.53*	(−0.54*)
Cognitive Estimates	−0.67**	(−0.52*)	−0.53*	(−0.32)	−0.22	(−0.22)
Picture Arrangement errors	−0.10	(0.07)	−0.49*	(−0.41)	0.39	(0.44*)
Alzheimer patients (n = 16)						
FAS Verbal Fluency	0.36	(0.27)	0.40	(0.30)	0.15	(0.11)
Miller Correction	0.00	(−0.12)	−0.24	(−0.42)	0.18	(0.15)
Birds and Colours	0.57*	(0.60**)	0.79***	(0.86***)	0.12	(0.12)
Modified Weigl	0.34	(0.23)	0.51*	(0.39)	0.05	(−0.01)
Card-sorting categories	0.53*	(0.46*)	0.51*	(0.40)	0.29	(0.27)
Card-sorting % perseverations	−0.26	(−0.25)	−0.26	(−0.26)	−0.13	(−0.12)
Cognitive Estimates	−0.63**	(−0.57*)	−0.54*	(−0.42)	−0.39	(−0.40)
Picture Arrangement errors	−0.06	(0.01)	−0.43*	(−0.39)	0.26	(0.29)

* $P < 0.05$, 1-tailed, ** $P < 0.01$, 1-tailed; *** $P < 0.001$, 1-tailed. Figures in parentheses = correlations after partialling for IQ

personalities test; and to 'recent' facts/events on the personal semantic and autobiographical schedules. In Table 6, the correlations shown for 'early' and 'more recent' memories are the mean correlations across the 5 remote memory tests. The third column in this table shows the statistical significance of the difference between the correlations with 'early' and 'more recent' memories respectively. Results are given for the 5 frontal tests which, in the Korsakoff group, correlated significantly with either RMQ or R/A difference scores.

Table 6 shows that the mean correlations with (raw) scores for 'early' and 'more recent' items were generally lower than the correlations with the overall RMQ. It also shows that in the Alzheimer and the total patient groups, there were no significant differences in the sizes of the correlations of 'early' and 'more recent' memories with frontal tests, IQ or AMQ. In the Korsakoff group, only IQ and cognitive estimates' score correlated to a significantly greater extent with 'early' than with 'more recent' memories, although the significant difference for cognitive estimates disappeared after partialling for IQ. FAS verbal fluency and card-sorting categories correlated significantly more highly with 'recent' than with 'early' memories, possibly reflecting the fact that 'early' memories were relatively preserved in the Korsakoff group, who showed a steeper temporal gradient than Alzheimer patients (Kopelman, 1989). In summary, frontal test performance in the Alzheimer group correlated to a similar degree with 'early' and with 'more recent' items on the retrograde tests. In the Korsakoff group, there was a trend whereby frontal test performance (except at cognitive estimates) was correlated to a somewhat greater extent with scores at 'more recent' rather than 'early' items, possibly reflecting the steeper temporal gradient in this group, whereas IQ correlated more highly with memory for 'early' items.

Table 7 shows the full matrix of the correlations between the individual remote memory tests and the

TABLE 6. CORRELATIONS OF FRONTAL TESTS, IQ, AND AMQ WITH 'EARLY' AND 'MORE RECENT' MEMORIES ON RETROGRADE TESTS, AND THE STATISTICAL SIGNIFICANCE OF DIFFERENCES BETWEEN THE CORRELATIONS

	'Early' memories	*'More recent' memories*	*Significance of difference between correlations (2t)*
Total patients (n = 32)			
FAS Verbal Fluency	0.35*	0.35*	n.s.
Birds and Colours	0.37*	0.34*	n.s.
Card-sorting categories	0.31*	0.29	n.s.
Card-sorting % perseverations	−0.22	−0.29	n.s.
Cognitive Estimates	−0.47**	−0.36*	n.s.
Full-scale IQ	0.44**	0.19	n.s.
AMQ	0.28	0.37*	n.s.
Korsakoff patients (n = 16)			
FAS Verbal Fluency	0.31	0.50*	<0.05
Birds and Colours	0.18	0.36	n.s.
Card-sorting categories	0.06	0.28	<0.05
Card-sorting % perseverations	−0.14	−0.32	n.s.
Cognitive Estimates	−0.55* (−0.34)	−0.29 (−0.26)	<0.05
Full-scale IQ	0.49*	0.15	<0.01
AMQ	0.35	0.43*	n.s.
Alzheimer patients (n = 16)			
FAS Verbal Fluency	0.31	0.16	n.s.
Birds and Colours	0.38	0.34	n.s.
Card-sorting categories	0.34	0.33	n.s.
Card-sorting % perseverations	−0.19	−0.23	n.s.
Cognitive Estimates	−0.41	−0.48*	n.s.
Full-scale IQ	0.28	0.18	n.s.
AMQ	0.43*	0.41	n.s.

* $P < 0.05$, 1-tailed; ** $P < 0.01$, 1-tailed. Figures in parentheses = correlations after partialling for IQ.

TABLE 7 CORRELATIONS BETWEEN INDIVIDUAL RETROGRADE (REMOTE MEMORY) TESTS AND INDIVIDUAL FRONTAL TESTS: TOTAL PATIENT SAMPLE (n = 32)

	News event recognition	*News event recall*	*Famous personalities*	*Personal semantic schedule*	*Autobiographical incidents schedule*
FAS Verbal Fluency	0.41**	0.45**	0.39*	0.34*	0.36*
Miller Correction	0.23	0.14	0.00	−0.01	0.07
Birds and Colours	0.43**	0.43**	0.19	0.38*	0.62***
Modified Weigl	0.16	0.27	0.04	0.31*	0.38*
Card-sorting categories	0.41*	0.47**	0.09	0.33*	0.49**
Card-sorting % perseverations	−0.43**	−0.27	−0.15	−0.30*	−0.24
Cognitive Estimates	−0.51**	−0.49**	−0.59***	−0.50**	−0.36*
Picture Arrangement errors	−0.15	−0.19	−0.18	−0.20	−0.15

* $P < 0.05$, 1-tailed, ** $P < 0.01$, 1-tailed, *** $P < 0.001$, 1-tailed.

individual frontal tests in the total patient group. Although the personalities test (which depends on familiarity judgements) showed fewer significant correlations with the frontal tests, there did not appear to be any overall difference between the other remote memory tests in terms of the strength of their association with frontal test performance.

TABLE 8. FRONTAL AND CT SCAN CORRELATIONS

	RMQ	*Nonverbal short-term forgetting*[1]	*Verbal short-term forgetting*[2]
FAS Verbal Fluency	+0.52**	−0.07	Not available
Card-sorting % perseverations	−0.40*	−0.11	Not available
CT: VBR	−0.18	−0.68**	−0.51**
CT: cortical atrophy	−0.14	−0.54*	−0.52**
	(n = 32)	(n = 12)	(n = 28)

* $P < 0.05$, 1-tailed; ** $P < 0.01$, 1-tailed. [1] Data from Corsi block retention task (Kopelman, 1990) [2] Data from Brown-Peterson task (Kopelman, 1985).

Table 8 contrasts the results obtained for RMQ and 2 measures of 'short-term' forgetting within combined Korsakoff and Alzheimer groups in terms of correlations with 2 of the frontal tests and the 2 CT scan measures of cerebral atrophy. The findings indicate that remote memory performance is correlated significantly with frontal function rather than cortical atrophy, whereas impaired performance on the nonverbal 'short-term' forgetting task is related to the degree of cortical atrophy rather than frontal dysfunction.

Finally, a stepwise multiple regression was conducted to determine, within the total patient group, what proportion of the variance in RMQ could be accounted for by AMQ, IQ, and frontal test performance. The frontal tests included in the regression analysis were those which correlated significantly with RMQ after partialling for IQ (FAS, Birds and Colours, Card-sorting categories, and Cognitive Estimates). It was found that AMQ, IQ, and these 4 frontal tests could account for 65% of the variance in RMQ. By comparison, the correlation between RMQ and AMQ was only 0.45, indicating only 21% of shared variance. Moreover, the regression equation

$$\text{RMQ} = -1.64a + 1.13b + 0.37c + 67.4$$

accounted for 64% of the variance in RMQ, where a = cognitive estimates score, b = birds and colours score, and c = FAS score. In the Korsakoff and Alzheimer groups, taken separately, these 3 frontal tests accounted for 68.5% and 57% of the variance in RMQ, respectively.

Discussion

In a previous paper (Kopelman, 1989), it was argued that structural and/or neurochemical lesions within Mishkin's (1978) limbic circuits produce anterograde amnesia with or without a brief (temporally-limited) retrograde loss, e.g., in mild/moderate head injury (Russell and Smith, 1961), localized thalamic and/or mamillary body damage (Winocur *et al.*, 1984; Squire *et al.*, 1989), bilateral damage to the CA1 region of the hippocampus (Zola-Morgan *et al.*, 1986), and some cases of transient global amnesia (Hodges and Ward, 1989). Moreover, there is evidence that cholinergic depletion within basal forebrain projections to those circuits would be expected to produce anterograde amnesia without any retrograde impairment (Irle and Markowitsch, 1987; Kopelman and Corn, 1988). The temporally-extensive retrograde amnesia seen in the Korsakoff syndrome and Alzheimer-type dementia (Sagar *et al.*, 1988*b*; Kopelman, 1989) must therefore result from some superimposed 'cortical' pathology. A similar argument has recently been put forward by Dall'Ora *et al.* (1989). Those authors found that patients who had focal amnesia with evidence of 'widespread' cortical pathology (sometimes evident on CT scan, but sometimes not) performed significantly worse at a test of autobiographical memory than patients who lacked widespread cortical pathology. An apparent discrepancy between the results of the two studies—Kopelman (1989) obtained a pronounced temporal gradient for autobiographical memory, Dall'Ora *et al.* (1989) did not—was a consequence of the differing time-periods within which subjects were constrained to produce 'recent' memories (1–5 yrs versus 15 yrs): when the same technique was employed, identical results were obtained (S. Della Sala, personal communication).

However, a temporally-extensive loss of retrograde memories appears not to be a simple function of the presence of generalized cortical atrophy. In the Dall'Ora *et al.* (1989) study, a combination of radiological and qualitative judgements was made to assess the presence of 'widespread' pathology, and some of the subjects in this group had normal CT scans. In the present study, correlations of RMQ with the quantified CT scan assessments of VBR and 'cortical atrophy' were only −0.18 and −0.14, respectively, in the total patient sample.

With regard to frontal atrophy or dysfunction, a computerized CT scan measure of frontal *atrophy* (Baldy *et al.*, 1986) was not predictive of remote memory performance on most of the remote memory tests. However, there was a trend for those patients with little or no frontal atrophy to show a better response to the cues provided by recognition testing (relative to recall performance) on the news events test than those patients who showed a substantial degree of frontal atrophy on CT scan (Kopelman, 1989). Moreover, the present study indicates that tests of frontal *function* do appear to be predictive of performance on tests of retrograde amnesia in these two patient groups. In the Korsakoff group, the degree of frontal dysfunction was correlated with the severity of retrograde but not anterograde memory loss; whereas, in the Alzheimer group, frontal dysfunction was associated with both retrograde and anterograde impairment. Within the total patient group, there was a suggestion of a double dissociation with findings for nonverbal 'short-term' forgetting in terms of the relative importance of frontal dysfunction and general cortical atrophy for impairment on these tests. Moreover, a regression equation based on 3 of the frontal tests (FAS, Birds and Colours, Cognitive Estimates) predicted 64% of the variance in RMQ, whereas AMQ accounted for only 21% of the variance.

In the Korsakoff group, anterograde amnesia can be assumed to have resulted from focal diencephalic lesions (Mair *et al.*, 1979; Mayes *et al.*, 1988). The present findings suggest that the extensive retrograde loss may be a consequence of the frontal neuronal loss which is now known to occur commonly in this disorder (Harper *et al.*, 1987). In the Alzheimer group, in which the severity of retrograde and anterograde impairment was fairly uniform (Table 4), it can be assumed that there were widespread histological and neurochemical abnormalities (Tomlinson and Corsellis, 1984; Rossor and Iversen, 1986). Consequently, the correlations between RMQ and frontal function, obtained in both patient groups, may reflect a specific association, whereas the correlation between AMQ and frontal function, which was present only in the Alzheimer group, may be an artefact of the widespread neuropathology and cognitive impairment in this group.

Elsewhere, it has been argued that the substantial improvement which Korsakoff and Alzheimer patients show to cues provided by recognition testing on remote memory tests (Kopelman, 1989) argues for a retrieval deficit in retrograde amnesia (*cf* Weiskrantz, 1985). This may be accentuated by a progressive anterograde impairment in the Korsakoff group during the period of their heavy drinking, resulting in their particularly steep temporal gradient (Kopelman, 1989). Various other authors have argued that the frontal lobes play an organizational, supervisory, or 'executive' role in memory processes (e.g., Luria, 1973; Warrington and Weiskrantz, 1982), and the present finding that the correlation between frontal tests and 'early' or 'more recent' memories did not differ significantly is consistent with a deficit in the initiation, planning or organization of retrieval processes. One possibility would be that frontal dysfunction produces a failure to make use of the contextual cues necessary to retrieve or recollect memories from the distant past (Baddeley, 1982). However, such a hypothesis needs to be interpreted with caution in view of the finding that various aspects of contextual memory impairment appear to be only weakly related to frontal dysfunction (Kopelman, 1989; Pickering *et al.*, 1989; M. A. Shoqerat and A. R. Mayes, unpublished results). Consistent with the present argument, Baddeley and Wilson (1986) have postulated that the frontal lobes are involved in the initiation, control, and evaluation of memory search. Similarly, Stuss and Benson (1986) argued that 'frontal lobe damage does not interfere with putting material into storage', but does affect 'organisational . . . processes [in] memory' '. . . including attention, motivation, programming, regulation and verification'. Shallice's (1988) model would suggest that a frontal 'supervisory system' is required to specify and verify the retrieval of remote, episodic memories. Moreover, Tulving (1989) has recently reported increased frontal blood flow when 3 healthy subjects were asked to retrieve autobiographical memories.

It should be emphasized that it appears to be the *combination* of frontal dysfunction and limbic-diencephalic pathology which is necessary to produce a temporally-extensive retrograde loss, as isolated frontal lesions generally have relatively small effects on memory function (Stuss and Benson, 1986; Schacter, 1987; but see Baddeley and Wilson, 1986). In short, the effects of combined lesions are greater than the sum of isolated lesions, as also occurs in bilateral temporal lobe pathology (Milner, 1972). Moreover, recent descriptions of 2 patients with an extensive retrograde loss in the presence of apparently normal frontal function (Salmon *et al.*, 1988; Becker *et al.*, 1990) suggest that lesions elsewhere (e.g., the lateral temporal lobes) may sometimes provide an alternative pathway to the same effect.

It should also be noted that 'fantastic' or 'spontaneous' confabulation (Berlyne, 1972; Kopelman, 1987*b*) has been attributed by various authors to frontal dysfunction (Luria, 1976; Stuss *et al.*, 1978; Kapur and Coughlan, 1980; Baddeley and Wilson, 1986). It is intriguing to speculate that orbitofrontal lesions, associated with cognitive disinhibition, may produce 'spontaneous' confabulation (*cf.* Stuss and Benson, 1984), whereas dorsolateral lesions, associated with cognitive aspontaneity, may produce impoverished retrieval of remote memories. However, tests of frontal function do not discriminate well between orbitofrontal and dorsolateral lesions, and this hypothesis requires further investigation.

CONCLUSIONS

The present study has demonstrated that, in a sample of 32 patients, there were statistically significant intercorrelations between 3 'fluency' tests and 3 card-sorting tests, even though the degree of shared variance was relatively low. The relationship of another test—picture arrangement errors—to the other frontal tests was more equivocal, although performance did correlate significantly with the 'fluency' scores in the Alzheimer group. Performance at one other test—cognitive estimates—appeared to be unrelated to performance at the other 'frontal' tests. In view of the high correlation of performance at this test with measures of retrograde and anterograde memory, it is intriguing to suggest that it is a marker of some separate site of frontal pathology (e.g., in the orbitofrontal region, *see* Leng and Parkin, 1988), but it should be noted that cognitive estimates scores also correlated significantly with measures of IQ and general cortical atrophy.

In the present study, there was no evidence that variability in release from proactive interference was related to measures of frontal function in either Korsakoff or Alzheimer patients. This finding is consistent with recent observations that impaired memory for temporal and spatial context and for the modality of presentation of learned material, as well as impaired 'short-term' retention of nonverbal material, may be only very weakly related to frontal dysfunction in these patient groups (Kopelman, 1989; Pickering *et al.*, 1989; M. A. Shoquerat and A. R. Mayes, unpublished results). However, this finding is qualified by the observation that, in general, the patients in this study showed 'normal' release from PI. It appears that the conditions in which Korsakoff and Alzheimer patients show impaired release are tightly constrained.

On the other hand, it has been demonstrated that frontal dysfunction does appear to be related to the defective retrieval of retrograde memories in both patient groups (and is also correlated with the severity of anterograde impairment in the Alzheimer patients). It has been argued that structural or neurochemical lesions within limbic-diencephalic circuits produce anterograde memory impairment with or without a brief retrograde loss, whereas frontal dysfunction either produces or contributes to the temporally-extensive retrograde loss in these two groups of patients. It has also been suggested that, whilst orbitofrontal lesions may produce 'spontaneous' confabulation (disinhibited retrieval of past memories and associations), dorsolateral frontal lesions (occurring in combination with limbic-diencephalic pathology) may account for an impoverished retrieval of retrograde memories.

ACKNOWLEDGEMENTS

The present data were collected when the author was working in the Section of Neuropsychiatry at the Institute of Psychiatry on a Wellcome Trust Lectureship. The CT scan measure of frontal atrophy briefly mentioned in this paper refers to work conducted by Mr R. Baldy, Dr R. Jacobson, and Professor W. A. Lishman in that department. Data analysis has been conducted at Charing Cross Hospital using SPSS-PC. The author is grateful to the various consultants who allowed him to test their patients, and to Mrs Gill Sunderland and Miss Claire Hook for secretarial assistance.

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(Received August 15, 1989. Revised January 9, 1990. Accepted January 12, 1990)

Brain (1991), **114**, 139–153

LOCALIZATION OF THE PRIMARY AUDITORY AREA IN MAN

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SUMMARY

The localization of the primary auditory cortex in man was studied by direct recordings in 150 different sites in the superior transverse gyrus, especially in Heschl's gyrus and the planum temporale. The distribution of the primary evoked responses (N13/P17/N26) was studied in 15 epileptic patients who were candidates for surgical treatment. Precise topography of recording sites was determined stereotactically. Our results provide evidence for considering only a restricted portion of Heschl's gyrus (its posteromedial part) as the primary auditory area.

INTRODUCTION

Many studies in man have attempted to evaluate the different components of scalp auditory evoked potentials (Peronnet *et al.*, 1974; Picton *et al.*, 1974; Streletz *et al.*, 1977; Wood and Wolpaw, 1982) and have speculated on the cortical localization of their generators (Vaughan and Ritter, 1970; Wood and Wolpaw, 1982). But the scalp studies have failed to identify stable components at latencies compatible with the earliest cortical response to the thalamocortical volley or to identify precisely the structures activated prior to the N100 emergence (Sams *et al.*, 1985). This is because the auditory areas lie deep within the sylvian fissure and require depth electrodes for their detailed physiological exploration. Few depth studies have been undertaken in man. Chatrian *et al.* (1960) were the first to record evoked responses to clicks with multilead depth electrodes. They showed that the responses did not seem to be confined to the posterior part of the superior temporal gyrus. However, the limited number and the dispersion of their recording sites did not permit precise localization of the generators. Celesia and Puletti (1969) corroborated these results, indicating that in man the region responding to auditory stimulation included Heschl's transverse temporal gyrus, the posterior part of the superior temporal gyrus and the upper bank of the sylvian fissure. Although they considered that it was unlikely that this entire region represented the primary cortex, they were unable to make functional subdivisions of the auditory cortex because the surgical technique allowed only approximate anatomical localization of their recording sites. Identification of the responsive area was obtained by comparing their landmarks along the longitudinal axis of the sylvian fissure with those of dissected human brains; such a method could not take account of noticeable individual variations in size, situation and orientation of the gyri in the temporal lobe (Szikla *et al.*, 1977).

The presurgical exploration (stereoelectroencephalography or SEEG; Bancaud *et al.*, 1965) of patients with temporal, temporoparietal or temporofrontal epilepsy requires

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stereotactic implantation of multiple depth eletrodes in the temporal lobe and neighbouring areas of adjacent lobes in order to determine the accurate limits of future cortical excision (Talairach *et al.*, 1974). In addition to the cases where the posterior areas of the superior temporal gyrus (STG) are suspected of constituting the whole or part of the epileptogenic zone, this region is frequently explored because of its strategic position as a pathway for ictal discharge propagation towards inferior parietal and opercular regions (Bancaud and Chauvel, 1987), as well as for the necessity for functional mapping before surgery. We therefore had the opportunity of recording intracerebrally auditory evoked potentials in various sites in the STG, especially in Heschl's gyrus and the planum temporale. A three-dimensional reconstruction of electrode lead positions, as well as an indirect visualization of cortical structures, was made possible by the stereotactic angiography method (Szikla *et al.*, 1977): the precision of this method allows us to rely on individual anatomical correlates for an accurate interpretation of the physiological data.

This paper reports the results obtained for recordings of the early cortical auditory evoked potentials and delineation of the primary auditory area. Click stimulation was employed, its brief duration permitting precise measurement of evoked response latency. This technique helped in the identification of the components of the auditory evoked potential (AEP), particularly in discriminating early from late potentials. The investigation of middle latency evoked potentials and tonotopy of the human auditory cortex will be the subject of a second report.

PATIENTS AND METHODS

Recordings were performed in 15 adult patients (aged 16−38 yrs) with drug-resistant partial seizures of right (13 patients) or left (2 patients) temporal lobe origin. On the basis of conventional EEG and clinical observations, they were judged suitable for surgery. Each patient underwent a stereoelectroencephalographic exploration (SEEG) with chronic depth electrodes (fig. 1) as extensively described by Bancaud *et al.* (1965). The recordings were all from the nondominant hemisphere. All patients were informed about the research protocol to be followed during their SEEG and had given their consent. During recording sessions, they lay comfortably in a bed and were alert and cooperative.

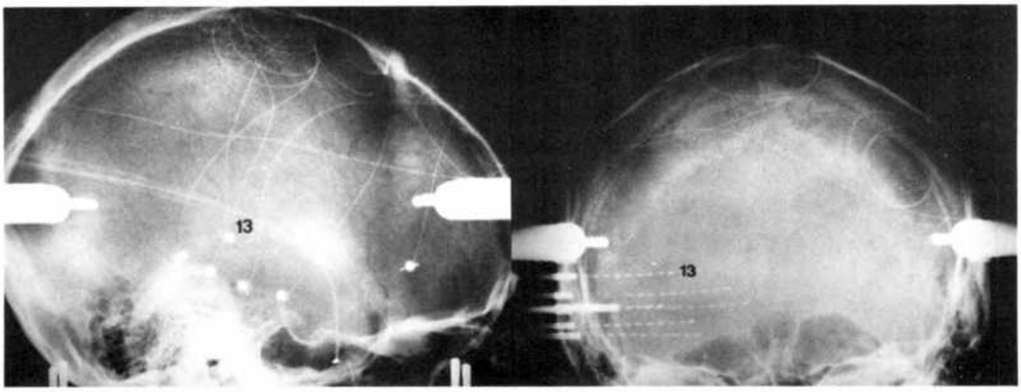

FIG. 1. *Case 13*. Stereotactic radiographic control film (lateral and anteroposterior view) at the end of SEEG implantation; the 13 on the fig. indicates the electrode exploring the right Heschl's gyrus.

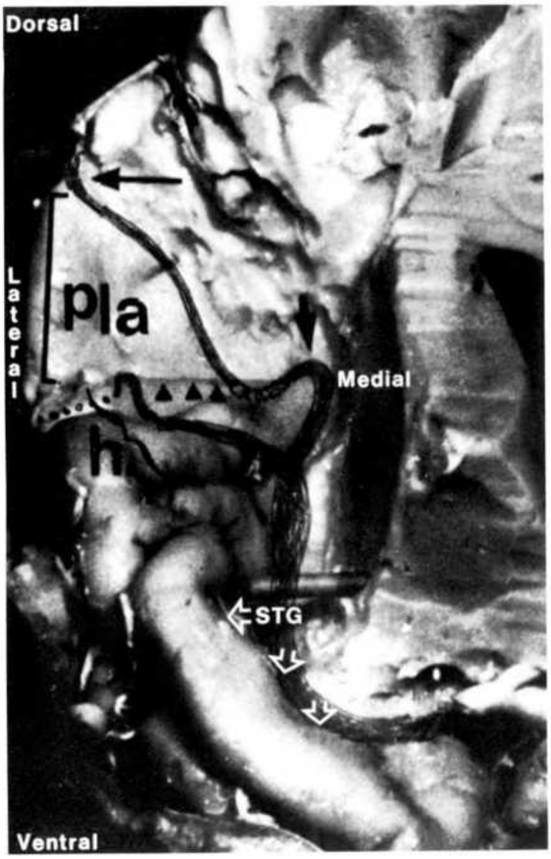

FIG. 2. Anatomical specimen after removal of the frontal and parietal opercula exposing the temporal lobe, Heschl's gyrus (h) and the planum temporale (pla) (posterior part of the superior temporal gyrus). The portion of the arterial branch between the two arrows also seen on angiography (*see* fig. 3) defines the position of the planum temporale: the short thick arrow marks the arterial loop running around the deep portion of Heschl's gyrus, the longer thin arrow the end point of the planum temporale and the arrowheads the transverse sulcus. White arrows indicate the anterior part of the superior temporal gyrus (STG). (From the Atlas of Szikla *et al.* (1977), adapted with permission.)

Anatomical definition of depth electrode position

The anatomical definition of the recording electrode site is based on a stereotaxic method which has previously been described elsewhere (Szikla *et al.*, 1977; Musolino *et al.*, 1988). With this 'in vivo' angiographic method for defining sulci and gyri, it is possible to identify the sylvian fissure, insular triangle, planum temporale (Pla), Heschl's gyrus (H) and the transverse sulcus which separates them. In fact, the most posterior insular branch of the middle cerebral artery forms a very characteristic loop around the medial portion of Heschl's gyrus. It then runs posteriorly and mediolaterally on the planum temporale and afterwards appears on the supramarginal gyrus (fig. 2). The initial segment of this arterial loop corresponds to the most posterior point of the insular triangle. This allows angiography to identify Heschl's gyrus and the planum temporale precisely (fig. 3).

In this study, the three-dimensional position of each electrode lead was defined and measured in relation to this arterial branch. Fig. 4 shows the position of a given electrode. The tridimensional coordinates are as follows: (1) distance from the posterior point of the insular triangle, representing the deepest point of Heschl's gyrus (the anteroposterior coordinate); (2) distance from the sylvian fissure (the dorsoventral coordinate); (3) distance from the insular plane (the mediolateral coordinate). From these coordinates, we were able to classify cases as a function of the anatomical location of the electrode (Table 1).

Electrode tracks are visible on MRI examination performed after the SEEG, making it possible to verify the electrode location (fig. 5). Moreover, an original method of comparative study of stereotactic stereoscopic angiography and MRI examination allows us to collect more detailed data on the spatial organization of sulci and gyri. Magnified sagittal MRI images are matched against angiographic films using ventricular bicommissural distances and/or vascular landmarks visible both in the MRI and stereotactic angiography (Musolino *et al.*, 1988; Talairach and Tournoux, 1988).

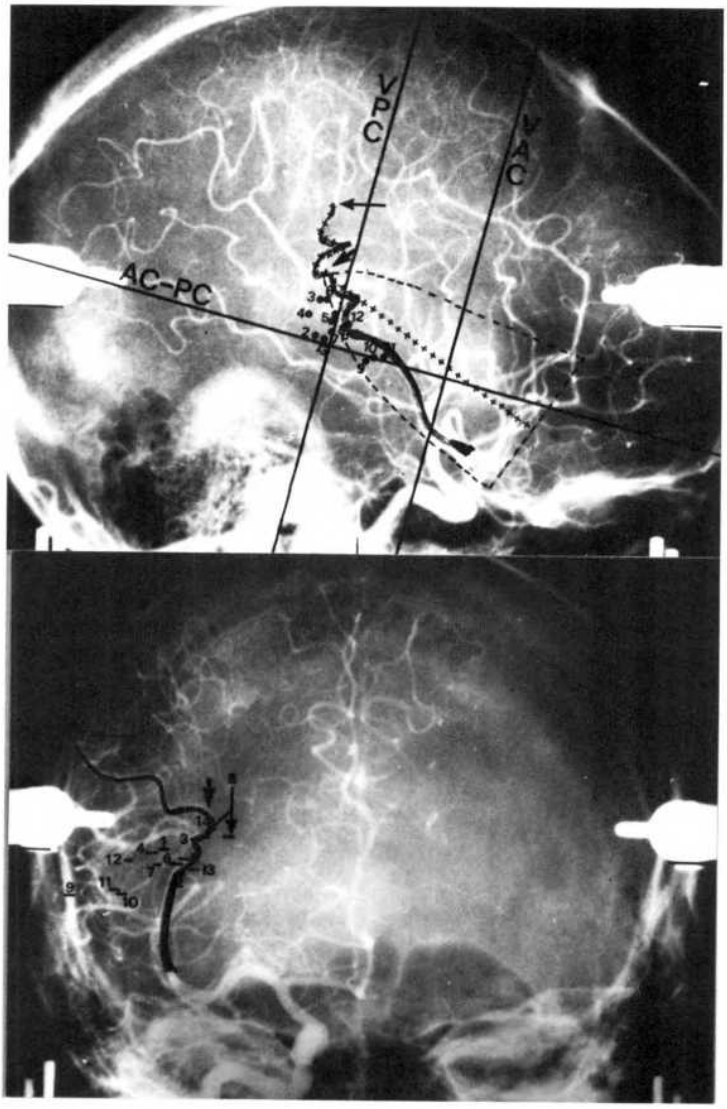

FIG. 3. *Case 13.* Stereotactic angiography with lateral (*upper panel*) and anteroposterior (*lower panel*) views. A middle arterial branch from the level of the insula loops around the deep portion of Heschl's gyrus (lower arrow) then runs around the planum temporale (arterial segment between the two arrows). The 'more responsive' leads of the electrode exploring the posterior portion of the STG in this case are transposed onto the angiogram, the dimensions of which approximate to average for all cases. Transposition is made in relation to the position of the arterial loop around Heschl's gyrus and to the sylvian fissure. The numbers indicate the recording sites for each case. AC-PC = bicommissural line transposed from the ventriculographic examination; VAC = vertical line to the anterior commissure; VPC = vertical line to the posterior commissure.

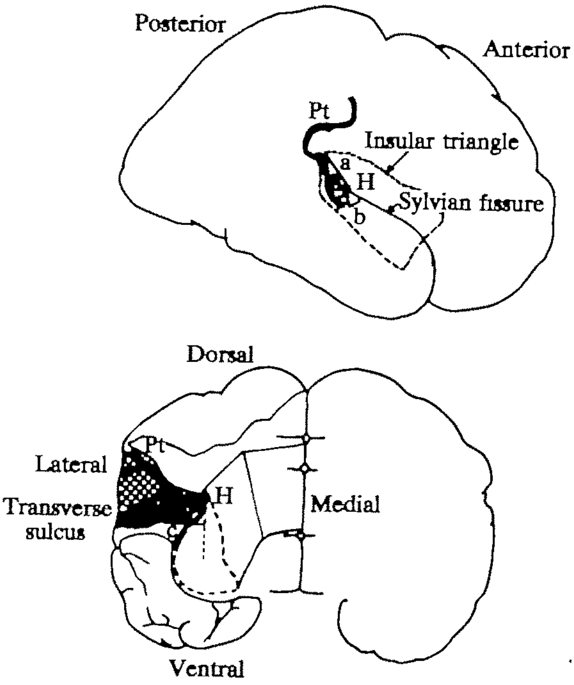

FIG. 4. Anatomical localization (schematic) of an electrode (☆) exploring Heschl's gyrus. The method for reconstruction of its position in the three-dimensional coordinates (a, b, c) is explained in the text. In this diagram, the location of Heschl's gyrus and the planum temporale in relation to the sylvian fissure and insular triangle (lateral and anterior views) are indicated. Cross-hatched area = planum temporale; dashed area = Heschl's gyrus; dotted lines enclose the insular triangle; Pt = arterial segment of middle cerebral artery outlining the orientation of the planum temporale; H = arterial segment outlining the orientation of Heschl's gyrus.

TABLE I. LOCALIZATION OF ELECTRODE POSITIONS

Anatomical localization in Heschl's gyrus	*Cases*
Dorsoposteromedial part	1, 3, 5, 8, 14
Dorsoposterointermediate part	6
Dorsoposterolateral part	12
Dorsoanterolateral part	10, 11
Ventroposteromedial part	13
Ventroposterointermediate part	2, 4, 7, 15
Ventroanterolateral part	9

Auditory stimulation

The sounds used were rarefied clicks (0.1 ms duration) generated by a SM 700 Nicolet auditory stimulator and delivered to the right and/or left ear through TDH 39 earphones at a rate of 5.1/s. Auditory threshold was determined for each patient and the stimulus intensity adjusted to 70 dB SL (sensation level).

The effect of stimulus intensity on AEPs was studied in 3 patients (Cases 4, 6, 13). Binaural stimulation (at a rate of 5.1/s) was delivered at intensities decreasing from 70 dB SL to the auditory threshold. The corresponding amplitudes and latencies of the AEPs recorded from the different leads of the depth electrodes were analysed as a function of intensity. In addition, the effect of stimulus rate on AEPs was studied in Cases 6 and 12; 70 dB SL clicks at varying rates (5.1; 9.1; 14.1; 19.1/s) were presented binaurally.

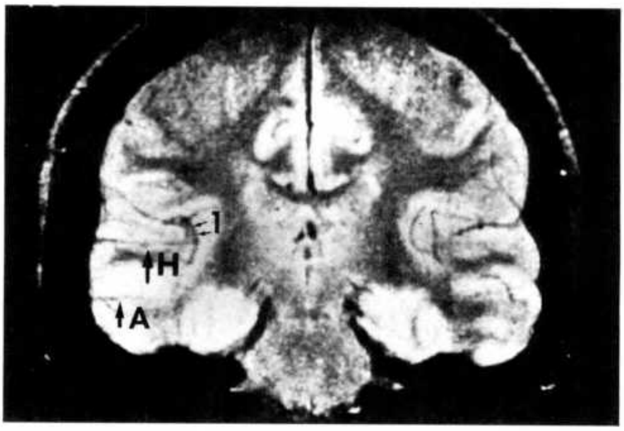

FIG. 5. MRI frontal cut (T1 weighted; TR: 425; Te: 12) from *Case 1*) performed 8 mos after removal of electrodes. 1 = arterial branch running on the planum temporale; H = track of the electrode that explored Heschl's gyrus; A = track of an electrode that explored the amygdaloid complex and was situated in the same frontal plane as electrode H.

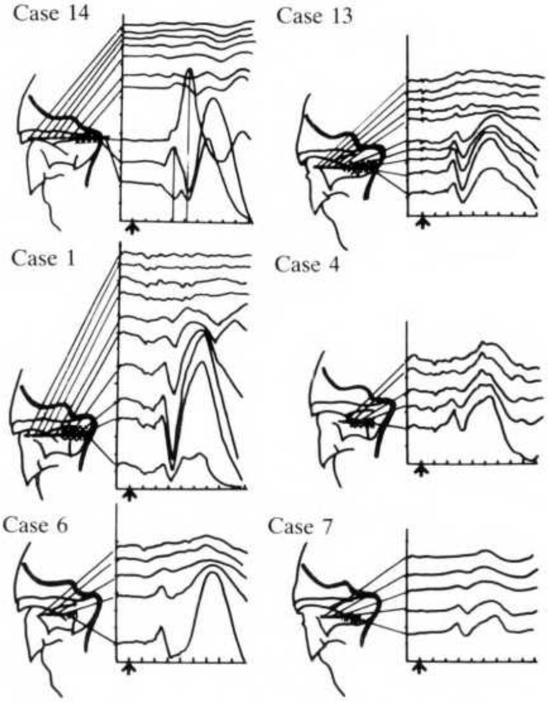

FIG. 6. Early AEP voltage distributions recorded from different sites of the right Heschl's gyrus electrode in 6 patients (*Cases 14, 1, 6, 13, 4, 7*). The schematic position of the electrode exploring Heschl's gyrus is represented on the left. Crossed areas show the responsive area. Each trace, on the right, corresponds to the monopolar recording of each lead of one electrode referenced to an extracephalic site. Note the polarity inversion in Case 14 (*see* text). Amplitude (6.25 μV/div) and time (5 ms/div) calibration at lower right. Arrows correspond to the auditory stimulation.

Intracerebral stimulation

In the course of every SEEG exploration, central electrical stimulation is regularly undertaken in order to confirm the anatomical localization of the electrodes, to perform functional mapping, to trigger seizures and to analyse preferential pathways of propagation (Bancaud *et al.*, 1968; Buser and Bancaud, 1983). We have taken this opportunity to study in particular the functional connections between Heschl's gyrus and other cortical or limbic areas, so far as the distribution of electrodes permitted. Bipolar shock stimuli were delivered between successive adjacent leads of each electrode. The parameters of stimulus were as follows: 100 μs duration, 5 mA intensity at a rate of 0.7/s. Evoked responses were recorded monopolarly on the leads of each other available electrode. Only data from STGs not included in the epileptogenic zone are reported.

Recording and averaging of intracerebral potentials

Intracerebral auditory evoked potentials were recorded from every lead of each depth electrode, all referenced to an extra cephalic reference looped around the neck. They were simultaneously amplified and filtered (15 to 1.5 kHz). The time base was 50 ms with a prestimulus time of 10%, i.e., electrical activity was averaegd 5 ms before and 45 ms after the stimulus. Each trial consisted of 1000 samples and 2 trials were obtained for each study. Evoked responses to intracerebral stimulation were recorded with the following parameters: 5 Hz–3 kHz, 10 samples. The polarity nomenclature used is such that negative potentials are up and positive down. Recordings of brainstem evoked potentials made before the SEEG confirmed that all the patients studied had normal afferent pathway conduction.

RESULTS

Recordings were performed in 15 patients from different sites of lateral (Heschl's gyrus (H), superior temporal gyrus (STG), intermediate temporal gyrus (ITG)) and medial (hippocampus, amygdala, parahippocampal gyrus) temporal lobe structures. In spite of these extensive recordings, early auditory evoked potentials could be recorded only from a limited area, localized in the posterior part of Heschl's gyrus. The large number of trials in each average resulted in a noise level below 2 μV. Thus the absence of a response implies that any volume-conducted activity was below this level.

The responses recorded in 6 patients from electrodes in different locations in the right Heschl's gyrus (*see* Table 1) are presented in fig. 6. They generally consisted of a triphasic potential whose components were labelled according to their polarity and mean peak latency. The individual values of different peak latencies for each patient are given in Table 2; accordingly, the typical early AEP sequence was an N13–P17–N26 potential. The onset of the response varied between 8 and 10 ms. Of the 2 patients with implantation on the left, we recorded early auditory evoked potentials in only 1 (Case 15). Although the peak latencies were somewhat delayed (N16, P23, N36) in this patient, it is not possible to assume that an interhemispheric latency difference reflects anatomical asymmetries (Geschwind and Levitsky (1968) and Galaburda *et al.* (1978)) rather than an insufficient sampling of left STG recordings. This is why values for Case 15 are tabulated separately from the calculation of the means given in Table 2.

In most of the cases, the N13, P17 and N26 components were constant in polarity at all recordings, varying only in amplitude across anatomical sites. However, a remarkable polarity reversal of the first component (N13) was noted in Cases 8 and 14. In Case 14 (fig. 6) the electrode tip (lead 1) passed the white matter of Heschl's gyrus precisely through its medial aspect. Consequently, the deepest lead (i.e., lead 1) lies over the surface of Heschl's gyrus and the adjacent one (lead 2) lies inside the cortex.

TABLE 2 LATENCY TO PEAKS OF AEP COMPONENTS IN RIGHT HESCHL'S GYRUS*

	Case									
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*13*	*14*
N13	12.8	12.3	12.3	14	14.7	12	14.3	12	11.9	15
P17	16.5	16	15	17.6	17.1	16	17.6	16	15.7	21
N16	28	23	18	25.8	30.5	31	24.6	23	26.7	30

* Mean ± SD: N13, 13 ms ± 1 21, P16.8 ms ± 2 86; N26, 06 ms ± 1.91.

The AEP component at a latency of 13 ms was negative in lead 1 and positive in lead 2, where it attained its maximal amplitude. Polarity reversals of subsequent components (P21/N21 and N30/P30) occurred more laterally between lead 2 lying in the cortex and lead 3 presumably lying in the white matter underlying Heschl's gyrus.

When depth electrodes were situated in the dorsoposteromedial part of Heschl's gyrus (Cases 1, 3, 8 and 14), amplitudes of the potentials were larger than when they were in the ventroposteromedial or intermediate part of the same gyrus (Cases 4, 6, 13). In lateral leads of the same electrode, amplitudes declined so abruptly that it became difficult to identify early AEPs clearly.

Table 3 displays the maximal peak-to-peak amplitude (expressed in absolute values) of the P17/N26 recorded from the most responsive lead of each electrode in all patients. The results are presented according to electrode location as described in the previous section. Maximal amplitudes are clearly recorded in the dorsoposteromedial (Cases 1, 3, 5, 8 and 14) or intermediate (Case 6) part of Heschl's gyrus. Early responses were

TABLE 3 AMPLITUDES OF COMPLEX P17/N26 AS A FUNCTION OF ANATOMICAL LOCATION OF ELECTRODE IN HESCHL'S GYRUS

Location of electrode	*Case*	*Wave*	*Amplitude (μV)*
Dorsoposteromedial part	1	P16/N28	45
	14	P16/N28	28
	5	P17/N30	22
	3	P15/N18	21
	8	P16/N23	16
Dorsoposterointermediate part	6	P16/N31	21
Dorsoposterolateral part	12	No early wave	–
Dorsoanterolateral part	10 L	No early wave	–
	11	No early wave	–
Ventroposteromedial part	13	P16/N28	15
Ventroposterointermediate part	4	P17/N26	11
	2	P16/N23	10
	15 L	P23/N36	6
	7	P17/N25	4
Ventroanterolateral part	9	No early wave	–

L = left.

never evoked in the dorsoposterolateral part (Case 12) or in the anterior part of the gyrus (Cases 9, 10, 11). In the ventroposteromedial or intermediate part of the gyrus, responses had the same morphology but their overall amplitude decreased. A voltage gradient therefore exists, so that early auditory responses are maximal in the posterior and dorsal areas of the gyrus ('the horn'), disappear laterally, and decrease greatly ventrally.

Effect of stimulation laterality

Since most of the patients had depth electrodes implanted in the right hemisphere, responses to left ear stimulation were more regularly studied. In some patients, we had the opportunity of comparing the effects of monaural (left and right) versus binaural stimulation. Results obtained in 2 different patients are illustrated in fig. 7. Contralateral

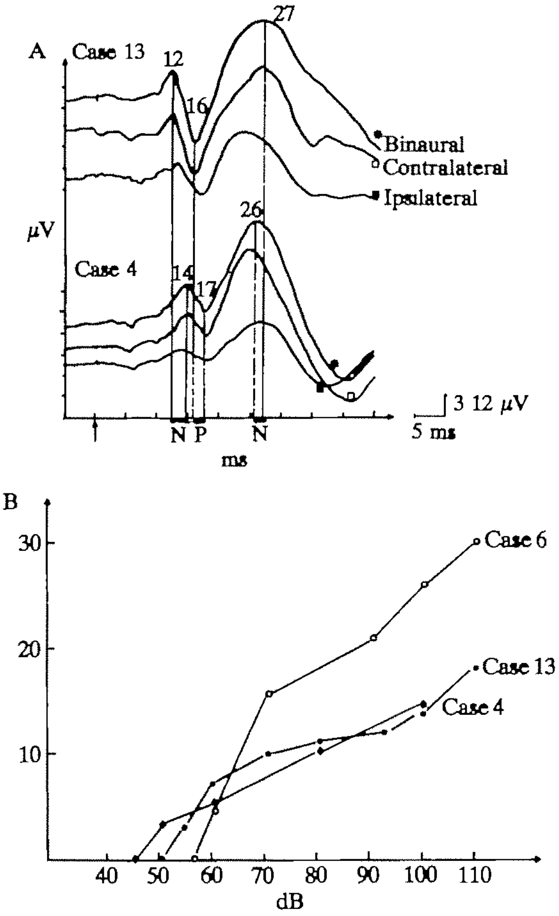

FIG 7. A, effect of monaural (ipsilateral and contralateral ear) and binaural stimulation on early EAPs in 2 cases Arrow indicates stimulus onset. B, effect of stimulus intensity on AEP amplitude in 3 cases. Stimulus intensity in dB SPL (sound pressure level) is represented on the x axis and AEP amplitude (in μV) on the y axis. The stimulus intensity at which no AEP was recorded (i e., the x-intercept of the 3 curves) was found to be equal to the perceptual threshold.

stimulation evoked a larger response than ipsilateral. In general, peak latencies did not change significantly as a function of the ear stimulated, although apparent latency shift could be occasionally observed (as in Case 13). AET amplitude was maximal for binaural stimulation, but its value never equalled an algebraic summation of the two monaural stimulation effects.

Effect of stimulus intensity

The peak-to-peak amplitudes of N13/P17 and P17/N26 complexes increased linearly with stimulus intensity. This is illustrated in fig. 7B for Cases 4, 6, 13 where P17/N26 amplitude is plotted against increasing intensity. The response completely disappeared when the patient could no longer hear the presented click. In every case tested, the objective AEP threshold corresponded to the subjective one.

Effect of stimulus rate

This effect on AEP amplitude was studied in Cases 6 and 12. In both, only components N13 and P17 followed the rate of stimulation up to 14.1/s. At 19.1/s, only N13 could be clearly identified as a frequency following response.

Intracortical connections of Heschl's gyrus

A single shock applied to the medial leads of H (localized in the dorsoposterior part of Heschl's gyrus) elicited a response in the medial leads of U (lateral part of the gyrus itself). Its latency (time from stimulation to onset of the first identifiable component)

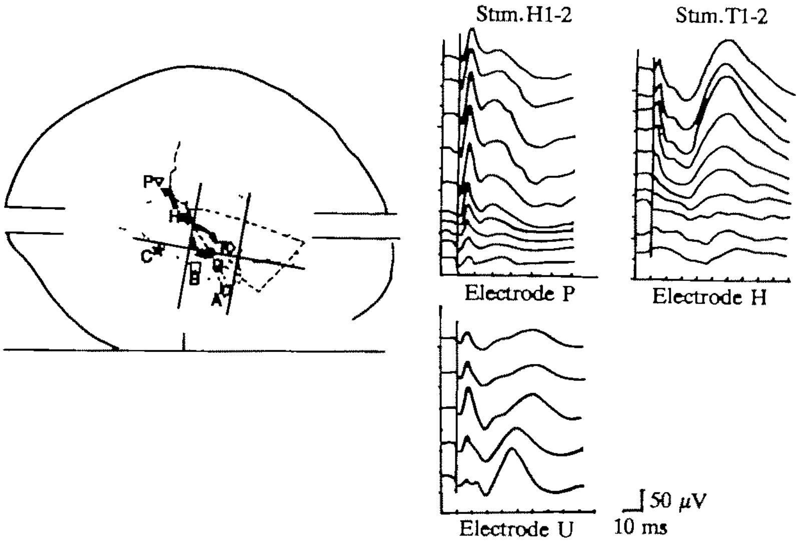

FIG 8 Intracortical connections of Heschl's gyrus. *Left*, schematic representation of relationship between the posteromedial part of Heschl's gyrus (H) and the planum temporale (P) and lateral part of the gyrus itself (T, U) indicated by arrows; no connection with amygdala (A), hippocampus (B) or parahippocampal gyrus (C) *Right*, evoked response voltage distribution along different electrodes as a function of the site of electrical stimulation. The most medial leads are at the bottom of each series of traces Bipolar stimulation of the mediolateral part of Heschl's gyrus (H1-2) evoked responses in P and U (*left column*). In contrast, stimulation of the lateral part of Heschl's gyrus (electrode T between leads 1 and 2) did not evoke a response in the mediolateral region of Heschl's gyrus (i.e., the 5 lower traces of H).

varied between 6 and 8 ms depending on the exact placement of the electrode. In the case illustrated (fig. 8, right), the evoked responses to H stimulation recorded in the medial leads of U (lateral part of Heschl's gyrus) and P (planum temporale) are in the same latency range. Responses recorded from P increase in amplitude from medial to lateral leads.

In the reverse situation, with T being stimulated and recording from H we never elicited a response from the inner part of the gyrus (medial leads); on the other hand, we observed late responses on the lateral leads which are localized in the planum temporale.

In summary, there is apparent connectivity from the dorsoposterior region to adjacent areas while the reverse is not observed.

DISCUSSION

The present work has attempted to delineate the area of human auditory cortex responsible for early generation of auditory evoked potentials. A precise comparison between the cytoarchitectonic subdivision of the transverse gyrus of Heschl and the physiological data needed to locate a primary sensory area was not previously available in man. Under the usual conditions of intraoperative recording, surface or depth electrodes can only be positioned remotely from the sites where the earliest cortical evoked potentials orginate. This is a result of the fact that Heschl's gyrus is deeply buried inside the sylvian fissure and is not directly visible after opening the dura mater during a neurosurgical operation.

The method developed at Ste Anne Hospital for epilepsy surgery includes a preoperative stage which delineates epileptogenic areas as well as providing information for the subsequent operative strategy. Such an SEEG exploration is achieved through stereotaxic implantation of several multilead electrodes. In cases of temporal lobe epilepsy, the posterior part of the STG is often explored in order to know about its possible involvement in seizures and/or to determine the posterior border of cortical excision. With respect to Heschl's gyrus, because of its obliquity in the transverse plane, these multilead electrodes, inserted orthogonally to the temporal convexity, explore either its outer or its inner part depending on their anterior or posterior placement in the STG.

Due to the aforementioned imposed conditions of remote recording, previous studies could not demonstrate more than the estimated position, along the anteroposterior axis of the sylvian fissure, of the early AEP generators (Celesia, 1976; Lee *et al.*, 1984). Moreover, precision as to their anatomical correlates was likely to be altered by major individual variations existing in that region. Thus, although we were using a reliable stereotactic method, the position of each electrode lead in the cerebral space had to be determined according to individual landmarks. A prior attempt, using the AC-PC bicommissural system (Talairach *et al.*, 1967) to calculate electrode lead coordinates on the basis of average dimensions of different brains in order to interpret physiological data in an 'average Heschl's gyrus', proved to lack the required precision. We have therefore considered the deep arterial loop around the most medial part of Heschl's gyrus as a region of common reference. This method enabled accurate anatomical localizations in the region of Heschl's gyrus and planum temporale, whatever the size and shape of individual brains.

The earliest cortical response is a negative wave with a mean latency of 13 ms followed by a positive wave (P17) and a negative one (N26). The overall amplitude of these components varies as a function of the recording location. They are maximal in the most posterior part of Heschl's gyrus and decrease in the dorsal to ventral and medial to lateral directions. These responses are absent when depth electrodes are located in the anterior or dorsolateral part of the gyrus.

In agreement with these data, polarity inversions were observed only in 2 patients (Cases 8, 14), precisely when the electrode passed through the cortex at the inner vertical part of the gyrus. This strongly suggests that the neuronal pool generating AEPs lies in the medial tip of Heschl's gyrus. This polarity inversion can be interpreted as a current dipole orientated orthogonally to the cortical surface. Our data do not necessarily restrict a generating neuronal pool to the medial wall of the Heschl's gyrus. AEP amplitude is also large and has a steep gradient in the adjacent horizontal part. However, due to the parallel trajectory of the recording electrode along the long axis of the gyrus, potentials arising from this part cannot be inverted across the successive electrode contacts.

The present hypothesis is strengthened by experimental data obtained in the monkey. Arezzo *et al.* (1975) showed that during recordings performed along depth electrodes implanted perpendicular to the horizontal plane of the STG, a polarity inversion of all AEP components was noted: initially surface-positive, they became negative when the electrode went through the cortex of the supratemporal plane. The voltage gradient of these primary responses was steep, such that a significant decrease was measured as the recording site moved a short distance away from the generator.

This interpretation is corroborated by the results of extensive exploration of the temporal lobe convexity in the neighbouring or on the lateral aspect of the transverse gyrus by two different recording techniques (Celesia and Puletti, 1969; Lee *et al.*, 1984). These studies obtained a response of comparable latency but inverse polarity: the earliest wave was positive (P1 latency range 12–19 ms). Its maximal amplitude recorded at the temporal lobe convexity, was nearly one-third less than in the present study. This can be explained by their electrodes being positioned at the cortical surface but remote from the generator, whereas our electrodes were in the cortex or in the white matter underneath the generator. Thus in the majority of our cases, an N13 was recorded, but when the electrode crossed the gyrus in such a way that one or two of the most medial leads were in fact situated on the other edge, i.e., on its surface side (Cases 8, 14), the polarity reversed to a P13.

The P17/N26 component appeared to originate from a region close to and overlapping with the source of the N13. In Case 14, it inverted approximately 3 mm laterally. Further away from the generator, these components could not be dissociated, so a triphasic N13/P17/N26 waveform was obtained, mirroring the P1/N1/P2 recorded at the cortical surface. Another distinctive feature arguing for N13 as the primary response was its frequency-following ability: whereas both the N13 and P17 followed stimulus rates up to 14.1/s, only the N13 was identifiable at 19.1/s.

The present data tend to define the primary auditory area as a restricted portion of Heschl's gyrus. When compared with the cytoarchitectonic data of Galaburda and Sanides (1980) in man, this dorsoposteromedial area would correspond to the koniocortex field, located in that part of Heschl's gyrus where polarity inversion of the earliest cortical

AEP component was observed. The boundaries seem to be superimposable on those that we have found, so far as the anatomical landmarks can be compared.

Similarly in the monkey, physiological studies followed by histological verifications (Arezzo *et al.*, 1975) indicate that the earliest cortical response (positive surface wave at 12 ms) arises from a limited region in the posteromedial part of the STP which centres on the cytoarchitectonically defined auditory koniocortex. The same is true for the following component, P22, which was mainly derived from this koniocortex. A similar organization was demonstrated in the cat (Kaga *et al.*, 1980) and in the guinea pig (Smith and Kraus, 1988), where the exploring electrode was implanted perpendicular to the cortical surface.

Amplitudes of the evoked responses were higher when stimuli were delivered to the contralateral than to the ipsilateral ear. They were maximal when the stimulation was binaural. These data are in agreement with previous studies in both man (Celesia, 1976) and animals (Rosenzweig, 1951) which suggested that each hemisphere receives input from both ears but that the contralateral ear is clearly better represented. Recordings at the level of the inferior colliculus in man showed that the amplitude of the ipsilateral response is smaller and more variable than the contralateral one (Møller and Jannetta, 1982). Another argument in favour of a predominant contralateral representation is given by direct stimulation of Heschl's gyrus in man: in our patients as well as in the cases reported by Penfield and Perot (1963), it elicited simple auditory hallucinations most frequently referred to the contralateral side.

Evoked response amplitude is a function of stimulus intensity as previously described by Teas and Kiang (1964) in the cat. We demonstrated here that the 'objective' auditory threshold measured by the stimulus intensity required to obtain a minimal cortical response corresponds to the 'subjective' perceptual threshold of the patient.

Organization of the responses to intracortical stimulation brings further indications for identification of this responsive area as a primary sensory area. Its stimulation evokes responses in the lateral part of Heschl's gyrus, the anterior part of the STG and in the planum temporale. Conversely, no response could be recorded in the posteromedial part of Heschl's gyrus to stimulation of any other adjacent region. Anatomical and physiological studies in the monkey (Bignall, 1969; Pandya *et al.*, 1969) have shown that the primary and the secondary auditory areas present different patterns of connections: the primary area mainly projects to associative areas and never to remote areas in the same hemisphere. In contrast, there are no connections from associative areas to the primary area. Thus the unidirectional connections demonstrated from posteromedial Heschl's gyrus to auditory associative areas provides converging evidence consistent with the experimental demonstration of primary auditory area connections in primates.

In conclusion, the present study performed by direct recording of auditory areas in man brings evidence for considering a restricted part of Heschl's gyrus, precisely its posteromedial part, as the primary auditory area.

ACKNOWLEDGEMENTS

We would like to thank Professor Eric Halgren for his suggestions and Janine Prevost for assistance with photography.

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(Received September 29, 1989. Revised January 12, 1990. Accepted January 23, 1990)

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IDIOPATHIC INTRACRANIAL HYPERTENSION

A PROSPECTIVE STUDY OF 50 PATIENTS

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SUMMARY

Management of patients with idiopathic intracranial hypertension (IIH) should be based on the presence and progression of visual loss. To characterize the clinical course of IIH more completely, we monitored the clinical status, especially visual function, in 50 consecutive newly-diagnosed patients over a period of 2 to 39 months (average follow-up 12.4 months). The mean age at onset of symptoms was 31 (range 11–58) yrs; 46 (92%) were women and 47 (94%) were obese (mean weight 90 kg). Common symptoms were headache (92%), transient visual obscurations (72%) and intracranial noises (60%); 13 of the patients (26%) initially had complaints of sustained visual loss. There was visual loss as determined by Goldmann perimetry in 96% and by automated perimetry in 92%. Contrast sensitivity testing was abnormal in 50% and Snellen acuity in 22%. Two patients (4%) became blind in both eyes. The Goldmann visual field grade improved in 60% of patients but visual function deteriorated in 5 (10%). Deterioration of visual field grade was significantly associated only with weight gain during the year before diagnosis. Visual loss in patients with IIH is common and is often reversible. Patients should be evaluated by perimetry using an appropriate strategy and contrast sensitivity testing, along with careful examination of the optic discs.

INTRODUCTION

Idiopathic intracranial hypertension (IIH) is a syndrome characterized by increased intracranial pressure with its associated signs and symptoms in an alert and oriented patient, but without localizing neurological findings. There is no evidence of deformity or obstruction of the ventricular system, and neurodiagnostic studies are otherwise normal except for increased cerebrospinal fluid (CSF) pressure. In addition, no secondary cause of raised intracranial pressure (ICP) is apparent (modified Dandy criteria for IIH; Smith, 1985).

The signs and symptoms of increased ICP are headache, with nausea and vomiting and intracranial noises; visual findings are diplopia due to sixth cranial nerve paresis, transient visual obscurations and papilloedema with its associated loss of sensory visual function.

Idiopathic intracranial hypertension was first described by Quincke (1897) and termed 'serous meningitis'. Nönne (1904) called it 'pseudotumor cerebri'. It was later referred to as 'otitic hydrocephalus' (Symonds, 1931), 'hypertensive meningeal hydrops' (Davidoff and Dyke, 1937) and 'toxic hydrocephalus' (McAlpine, 1937). The disorder became well delineated as a clinical entity in the 1940s when cerebral angiography with water soluble contrast agents was added to pneumoencephalography as improved techniques

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to diagnose cases of mass lesions. Foley (1955) introduced the term 'benign intracranial hypertension' and the large clinical study by Weisberg (1975) further defined this entity. It was not until the 1980s, however, that studies appeared that documented the high incidence of visual loss (Corbett *et al.*, 1982; Orcutt *et al.*, 1984; Wall *et al.*, 1987) and it became clear that the term 'benign' had to be discarded. Buchheit *et al.* (1969) suggested the term 'idiopathic intracranial hypertension' (IIH), which is more descriptive of the condition.

IIH is not rare. It occurs with a frequency of about 1 case per 100,000 population per yr or 19.3 per 100,000 in obese women aged 20–44 yrs (Durcan *et al.*, 1988). Loss of visual function is the only major form of morbidity associated with IIH. About 10% of patients develop bilateral blindness (Corbett *et al.*, 1982). Visual field loss is found with perimetry in 25–75% of eyes depending on the technique and strategy used (Wall and George, 1987).

Current knowledge of IIH is limited largely to information gained from retrospective studies. To date no prospective study has investigated visual loss in this disorder with a battery of sensitive visual testing techniques. Treatment decisions are based primarily on the presence and progression of visual loss. We therefore studied 50 consecutive patients to survey the clinical course of this disorder with an emphasis on visual loss, its change over time and factors leading to clinically detectable deterioration of visual function.

METHODS

Between 1982 and 1988, 73 patients with IIH were referred to the neuro-ophthalmology clinic at Tulane University Hospital; 12 were excluded from the analysis because they did not return for follow-up examination or were followed for less than 2 months. Two months was chosen as the lower limit of follow-up to maximize the number of cases and since, if a treatment has an effect in IIH, it is usually evident by 2 months. Six patients with previously documented papilloedema and elevated CSF pressure no longer had papilloedema at the time of their first study visit and were excluded; 5 additional patients were unreliable perimetry subjects and were also excluded. All patients met the modified Dandy criteria as defined above for the diagnosis of IIH. One patient had a previous enucleation in one eye for a melanoma but otherwise fulfilled these criteria.

At the initial visit, an 83 item history questionnaire was administered. A shortened form of the enquiry was given at follow-up visits Epidemiological data and symptoms that occur with IIH and other causes of raised ICP were included. A detailed medication history was also taken.

A neuro-ophthalmological examination was performed at each visit that included Snellen visual acuity and confrontation visual fields (finger, face and hand confrontation). Contrast sensitivity testing was performed with the Arden gratings for the first 2 yrs of the study. The methods used for this test can be found in a previous report (Wall, 1986).

The Vistech contrast test chart (Ginsberg, 1984) was used for the last 4 yrs of the study. Subjects viewed the correctly illuminated test chart from a distance of 3 m for spatial frequencies of 1.5, 3.0, 6.0, 12.0 and 18.0 cycles/deg. Using a four-alternative forced-choice paradigm for each spatial frequency, the contrast threshold was taken as the last patch in the series (least amount of contrast) for which the subject could correctly identify the grating orientation. Further details of our methods are found in another publication (Tweten *et al.*, 1990).

Colour vision testing was performed using fluorescent room illumination with both American Optical pseudoisochromatic plates and a colour comparison test. The colour comparison test was accomplished by having the patient view a red test object, alternately covering one eye. Any relative difference reported by the patient was recorded. Relative afferent pupillary defects were graded with neutral density filters (Thompson *et al.*, 1981). Ophthalmoscopic examination was performed at all visits. The degree of papilloedema was graded using Frisén's scheme (Frisén, 1982). Grade 0 defines an optic nerve head without swelling and grade 5 severe papilloedema. Stereoscopic fundus photos were taken on the initial visit and when change occurred.

Goldmann perimetry was performed at all visits by the same perimetrist (D.G.). The method used is shown in fig. 1. This modification of the Armaly-Drance strategy for glaucoma concentrates test loci where disc-related field defects occur.

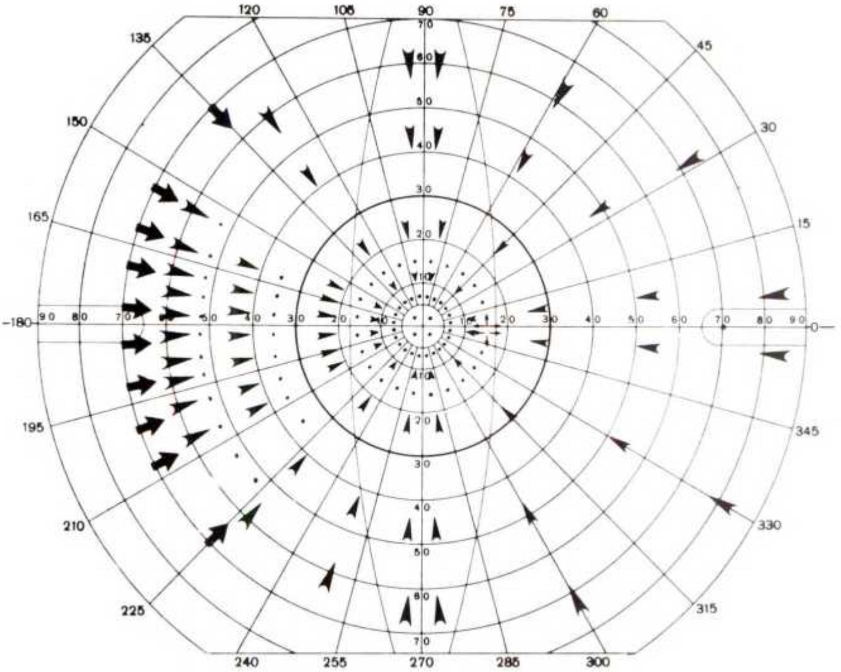

FIG. 1. Armaly-Drance visual field strategy used for Goldmann perimetry (right eye). Arrows indicate sites of kinetic testing, dots refer to stimulus loci of suprathreshold static testing.

Automated perimetry during the first 4 yrs of the study was performed with the Octopus 201 perimeter; for the last 3 yrs the Humphrey perimeter was employed. A full-field automated strategy was used for the first 17 of the 50 initial evaluations. Regardless of the perimeter, all other visual fields were performed with a program thresholding 72 points in the central 30° of the visual field; a 6° spaced grid, offset from the vertical and horizontal meridians was used. Of the 234 examinations, automated perimetry was unsuccessful 23 times for one of the following reasons: patients were unable to see the target, unreliable results occurred in a previously reliable patient or mechanical problems with the perimeter precluded the examination. The visual fields were graded for both types of perimetry using the criteria listed in the Appendix.

All patients had blood and CSF testing to exclude other disorders known to cause raised ICP. All patients also had CT brain scanning or MRI. If the results of these tests were abnormal, except for increased CSF pressure, the patient was excluded from the study.

Because of the small number of patients, a limited number of questions for statistical analysis were designated as study outcomes. These were as follows. (1) Is there a change in papilloedema grade and perimetry grade over time? (2) Does the severity of papilloedema grade correlate either with Goldmann or automated visual field grade? (3) Does the presence of transient visual obscurations correlate with visual loss on the initial examination as determined by Goldmann visual field grade? (4) Does the final optic cup size correlate with visual loss by Goldmann perimetry? (5) What factors are associated with deterioration of vision as determined by Goldmann visual field grade? Other statistical tests were performed for description and hypothesis generation.

Differences between groups were tested for statistical significance using a χ^2 test for nominal data. A Fisher's exact test was performed when the expected frequency in one of the groups was 5 or less. Two-tailed paired t tests were used for normally distributed quantitative data comparing the results of the initial and final examinations; the Wilcoxon test was used for corresponding nonparametric data. The independent t test (for parametric data) and the Mann-Whitney U test (for nonparametric data) were used for unpaired comparisons. The Spearman rank correlation was used as a measure of association for paired discrete qualitative data or data that were not normally distributed. When multiple tests were performed on a data set, the results were corrected using the Bonferroni adjustment for multiple statistical tests. The significance level was set at $P < 0.05$.

RESULTS

A total of 234 examinations was performed. Each patient was examined at least twice. The initial and final examinations of each patient were used for statistical analysis. The duration of follow-up ranged from 2 to 39 months; 15 were followed for 2–6 months, 9 for 6–12 months, 14 for 1–2 yrs and 12 for over 2 yrs. The average length of follow-up was 12.4 months.

Epidemiological data

Of the 50 patients in the study, 46 were women. The average age at onset of symptoms was 31 yrs (range 11–58 yrs; fig. 2); the average weight was 90 kg and 47 (94%) were obese. The averge weight gain over the 12 months before onset of symptoms was 7.7 kg. There was an average reported weight gain of 1.8 kg over the 2 months preceding the onset of symptoms.

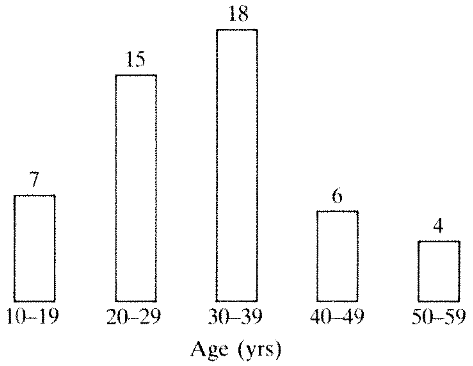

FIG. 2. Age by decade of patients in the study.

Of the 50 patients, 21 were evaluated directly from a population of a predominantly primary care facility (Charity Hospital of New Orleans). The remainder of the inception cohort was from a tertiary care referral practice and probably reflects selection bias for cases with more severe visual loss.

Thirty-one of the patients were black. Thirteen had a history of systemic arterial hypertension but only 4 were on antihypertensive medication at the time of their first visit. Three had diabetes mellitus. Ten patients had a history of iron deficiency anaemia and 3 were on replacement therapy with iron at the time of initial evaluation. One patient had hypothyroidism treated with physiological doses of thyroxine.

Medication history

Eleven patients were taking multiple vitamins in recommended amounts; none was taking supplemental vitamin A. Six patients were taking contraceptive pills. A case-control study of disease associations and symptoms of 38 of these patients is the subject of another report (Giuseffi *et al.*, 1991).

Symptoms

The frequency of the common visual symptoms and signs at the initial visit is given in Table 1 and fig. 3. Headache was reported by 36 patients as their first symptom; 6 first noted transient visual obscurations, 3 had visual loss, 3 experienced diplopia and 2 were asymptomatic. Thirteen of the patients (26%) reported sustained visual loss, with 10 relating loss in the right eye and 8 in the left eye. There was a significant relationship between the duration ($P = 0.04$) of sustained visual loss and visual field loss (Goldmann perimetry grade) on the initial examination. Visual loss also correlated with contrast sensitivity loss ($P = 0.02$). Nineteen patients (38%) noted horizontal diplopia, 36 (72%) had transient visual obscurations, 30 (60%) reported pulsatile intracranial noises, 27 (54%) noted sparkles (photopsias) and 46 (92%) had headache.

TABLE 1 FREQUENCY OF VISUAL FINDINGS IN PATIENTS AT THE INITIAL AND FINAL VISITS IN THIS SERIES*

Symptoms	*% patients*
Transient obscurations	72
Intracranial noises	60
Sparkles	54
Diplopia	38
Visual loss	26

	Patients		*Right eye*			*Left eye*		
Signs	*Init.*	*Final*	*Init.*	*Final*	*P*	*Init.*	*Final*	*P*
Papilloedema	100	64	100	62	<0.0001	100	64	<0.0001
Papilloedema > = Gr 2	64	38	68	30	–	70	28	–
Goldmann perimetry	96	60	86	50	0.001	90	54	0.0025
Goldmann grade > = 2	62	44	50	32	–	52	32	–
Automated perimetry	92	84	84	68	0.008	92	74	0.002
Automated grade > = 2	84	64	68	32	–	76	56	–
Contrast sensitivity	50	26	40	20	<0.0001	30	24	<0.0001
Colour comparison	40	24	–	–	–	–	–	–
Confrontation field	32	22	22	14	–	28	18	–
Choroidal folds	24	18	12	6	–	24	16	–
Snellen acuity	22	18	12	8	n.s.	14	12	n.s.
Colour plate score	16	10	16	10	0.002	14	10	0.04
Relative pupil defect	14	18	–	–	–	–	–	–
Sixth nerve palsy	12	0	–	–	–	–	–	–
Optic atrophy	4	6	1	2	n.s.	2	3	n.s.

* Init = initial examination, Final = final examination; > = Gr 2 = greater than or equal to Grade 2; n.s. = not significant. Involved or abnormal (%).

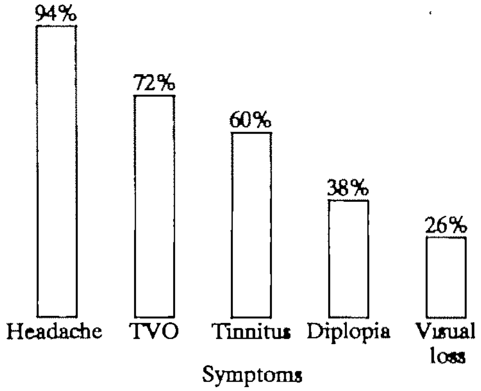

FIG. 3. Symptoms of patients with idiopathic intracranial hypertension (TVO–transient visual obscurations; tinnitus refers to patients reporting a pulsatile intracranial noise).

Headache. Of the 46 patients with headache, 39 reported the headache was pulsatile and 38 said the pain gradually increased in intensity. The headache was unilateral at times in 29 and lasted for longer than an hour in 41. Also, in 41 patients the headache was different from previous headaches; 44 of the patients that reported headaches said that their present head pain was the most severe of any they had experienced; 25 reported associated nausea and 19 noted vomiting. Twenty-eight had related neck stiffness and 9 had shoulder or arm pain; 29 stated that the headaches awakened them. Of the patients with headache, 35 (64%) had daily headaches.

Twenty-two patients had retrobulbar pain and 10 reported the pain occurred or worsened with eye movement. There was no significant relationship between the symptoms of retrobulbar pain, or pain on eye movements and loss of visual field at the initial or final examinations.

Transient visual obscurations. Transient visual obscurations were reported by 36 patients (72%). These were unilateral or bilateral episodes of visual loss usually lasting less than 1 min, followed by full recovery. The frequency of the obscurations ranged from rarely to many times a day. Most patients experienced several transient obscurations per week. Most obscurations lasted 5 to 30 s; 5 patients reported they lasted longer than 1 min. Rapidly assuming the standing position precipitated obscurations in 20 patients; 3 noted obscurations on lying down and 10 with bending, 4 reported that the Valsalva manoeuvre induced the transient visual loss and 3 had visual loss precipitated by glare or rapid change in illumination from dark to bright. The transient obscurations were gaze-evoked in 1 patient and were associated with headache in 2 patients. There was no relationship between the presence of transient visual obscurations and visual field loss (Goldmann or automated perimetry).

Sparkles (photopsias). These were reported by 27 patients (54%) and were described as white or less commonly coloured small flashes, flickering lights, stars twinkling, or flashes in the periphery of the visual field. The sparkles lasted for seconds and rarely lasted minutes. Their frequency ranged from once every several months to several times a day. There were no consistent provoking factors. There was no significant relationship between sparkles and visual field loss at the initial or final examination.

Intracranial noises. Pulsatile intracranial noises (pulsatile tinnitus) were reported by 30 patients (60%). They described the sound as being like 'a rushing river', a waterfall or a buzzing, whistling or blowing sound. Others said it sounded like 'water inside a balloon' or 'a rope whirling'.

Nineteen patients reported it was localized to one side of their head with 11 patients reporting a right-sided predominance; 7 noted a left-sided predominance and 1 described alternating laterality of the noise. Twelve of the patients reporting intracranial noises associated it with their headache. Most patients noticed it at night in a quiet room. The noise lasted from seconds to years, with most patients reporting a range of 1 – 10 min. The frequency varied from constant (3 patients) to rare; most patients noted noises daily to several times a week. There was no significant relationship between intracranial noises and loss of visual field at the initial or final examination.

Examination

The results of examination are summarized in Table 1. The best corrected Snellen visual acuity at the initial visit was worse than 20/20 in 12% of right eyes and 14% of left eyes. At the final visit 14% of right and 12% of left eyes were abnormal. Eleven patients (22%) at the initial visit and 13 (26%) at the final visit had abnormal Snellen acuity (fig. 4). There was no significant change in Snellen acuity from the first to the final examination in either eye. Although 5 patients had a worsening of the Goldmann perimetry visual field grade at their final visit, only 1 patient lost more than 2 lines of Snellen acuity. There was no significant correlation of change in Snellen acuity with change in the severity of papilloedema or with Goldmann visual field grade.

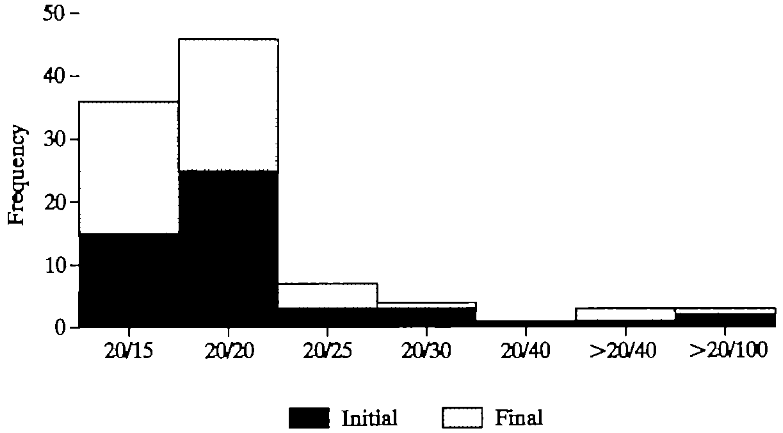

FIG. 4. Snellen visual acuity values at the initial (filled areas) and final (stippled areas) visits (measurement in worst eye used; >20/40 = acuity from 20/50 to 20/100; >20/100 = acuity worse than 20/100)

Twenty-five patients (50%) had abnormal contrast sensitivity at the initial visit; 20 patients had impairment in the right eye and 15 in the left. There was significant improvement of contrast sensitivity function ($P < 0.0001$); 62% had normal contrast sensitivity at the final visit. Of the 31 patients that had the Vistech contrast sensitivity evaluation, most of the visual loss was of the middle and high spatial frequencies. Two patients with contrast sensitivity testing that was initially normal had abnormal contrast sensitivity at the final visit. Contrast sensitivity loss was the only visual parameter that significantly correlated with the symptom of visual loss ($P = 0.02$).

Eight patients had pseudoisochromatic colour plate loss as defined by a score of 10 or less in at least one eye on their initial visit (14 plates used). There was a significant improvement between the initial and final colour plate scores ($P = 0.04$); 5 patients had abnormal scores on their final visit. A colour comparison test was abnormal in 20 patients at their initial visit and in 12 at the final visit.

Confrontation visual fields were abnormal in 32% of patients at the initial examination and 22% at the final examination (Table 2). The most common defects found were nasal loss, temporal loss (enlarged blind spot) and visual blurring. The results of perimetry are given in Table 3 and figs 5 and 6. Enlarged blind spots were only considered significant visual field defects when they encroached on the central 10° of the visual field. Goldmann perimetry was abnormal at the initial visit in 88 of 99 eyes (87%) and 51/99 (51%) at the final visit. By patients at the initial examination it was abnormal in at least one eye in 48 (96%). Grade 1 visual loss is minimal and unlikely to be noticed by the patient. With Goldmann perimetry, 31 patients (62%) had grade 2 loss or higher in at least one eye at the initial visit and 22 (44%) at the final visit. Two patients became blind (Goldmann grade 5) in both eyes; 3 developed blindness in at least one eye.

TABLE 2 RESULTS OF CONFRONTATION VISUAL FIELD EXAMINATION

	Right eye				*Left eye*			
Field defect	*Initial frequency*	%*	*Final frequency*	%*	*Initial frequency*	%*	*Final frequency*	%*
Normal	39	73.6	43	82.7	38	71.7	41	77.4
Nasal loss	4	7.5	1	1.9	4	7.5	2	3.8
Blurred	3	5.7	2	3.8	2	3.8	3	5.7
Constriction	2	3.8	1	1.9	2	3.8	1	1.9
Temporal loss	2	3.8	0	0	1	1.9	0	0
Central scotoma	1	1.9	1	1.9	1	1.9	1	1.9
Caecocentral scotoma	1	1.9	1	1.9	1	1.9	1	1.9
Sup altitudinal loss	1	1.9	1	1.9	0	0	0	0
Paracentral scotoma	0	0	1	1.9	1	1.9	0	0
Metamorphopsia	0	0	1	1.9	0	0	1	1.9
Sup. temporal loss	0	0	0	0	0	1.9	1	1.9
Temporal island	0	0	0	0	1	1.9	0	0
Less bright	0	0	0	0	1	1.9	1	1.9
Inf. nasal loss	0	0	0	0	0	0	1	1.9
Total	53	100	52	100	53	100	53	100

* % = percentage of total number of defects.

Automated perimetry was abnormal in 46 patients (92%) (*see* Table 3). By eyes, at the initial visit, automated perimetry was abnormal in 88 of 99 at the initial and 71 of 99 at the final visit; 42 patients (84%) had grade 2 loss or higher in at least one eye at the initial visit and 32 (64%) at the final visit.

The most common defects with Goldmann perimetry were constriction and inferonasal loss, followed by arcuate defects in the area surrounding fixation between 10 and 20°

TABLE 3 RESULTS OF PERIMETRY AT THE INITIAL AND FINAL EXAMINATIONS

	Goldmann perimetry							
	Right eye				*Left eye*			
Field defect	*Initial frequency*	%*	*Final frequency*	%*	*Initial frequency*	%*	*Final frequency*	%*
Normal	7	8.4	24	35.8	5	5.5	21	30.9
Enlarged blind spot**	26	31.3	13	19.4	39	42.9	17	25.0
Constriction	25	30.1	10	14.9	21	23.1	14	20.6
Inf. nasal loss	13	15.7	11	16.4	11	12.1	10	14.7
Sup. arcuate scotoma	3	3.6	3	4.5	4	4.4	0	0
Nasal loss	2	2.4	2	3.0	3	3.3	2	2.9
Inf. arcuate scotoma	2	2.4	1	1.5	2	2.2	0	0
Central scotoma	1	1.2	1	1.5	1	1.1	1	1.5
Sup. nasal loss	1	1.2	0	0	2	2.2	0	0
Inf. altitudinal loss	1	1.2	0	0	1	1.1	0	0
Sup. altitudinal loss	1	1.2	0	0	0	0	0	0
Sup. altitudinal loss	0	0	1	1.5	0	0	0	0
Inf. temporal loss	0	0	0	0	1	1.1	2	2.9
Paracentral scotoma	0	0	0	0	1	1.1	0	0
Temporal island	0	0	0	0	0	0	1	1.5
Not tested***	1	1.2	1	1.5	0	0	0	0
Total	83	100	67	100	91	100	68	100

	Automated perimetry							
	Right eye				*Left eye*			
Field defect	*Initial frequency*	%*	*Final frequency*	%*	*Initial frequency*	%*	*Final frequency*	%*
Normal	8	11.4	10	13.9	4	5.3	17	23.3
Constriction	19	27.1	10	13.9	19	25.0	14	19.2
Nasal loss	13	18.6	6	8.3	14	18.4	9	12.3
Inf. nasal loss	10	14.3	16	22.2	9	11.8	9	12.3
Enlarged blind spot	7	10.0	8	11.1	10	13.2	6	8.2
Caecocentral scotoma	3	4.3	2	2.8	2	2.6	1	1.4
Inf. arcuate scotoma	2	2.9	4	5.6	4	5.3	2	2.7
Sup. altitudinal loss	2	2.9	1	1.4	2	2.6	0	0
Sup. nasal loss	1	1.4	2	2.8	2	2.6	2	2.7
Central scotoma	1	1.4	0	0	1	1.3	0	0
Paracentral scotoma	0	0	2	2.8	2	2.6	2	2.7
Sup. arcuate scotoma	0	0	7	9.7	2	2.6	5	6.8
Sup. temporal loss	0	0	1	1.4	0	0	0	0
Inf. temporal loss	0	0	0	0	1	1.3	2	2.7
Inf. altitudinal loss	0	0	0	0	0	0	1	1.4
Cannot see target	2	2.9	3	4.2	2	2.6	3	4.1
Not tested***	2	2.9	0	0	2	2.6	0	0
Total	70	100	72	100	76	100	73	100

* Percentage of total number of defects. ** Blind spots plotted with Goldmann perimetry were commonly enlarged, classified as enlarged if clinically significant (defect encroached on central 10°). *** One eye enucleated for melanoma.

(Bjerrum area). The same defects were frequent with automated perimetry but in addition nasal loss was common (fig. 6). Only one visual field in one eye had a defect detected outside 30° with Goldmann perimetry not associated with a defect inside 30° on both Goldmann perimetry and automated perimetry.

There was significant improvement in Goldmann visual field grade from the initial to the final visit ($P = 0.002$); 60% of patients improved while 10% worsened. With

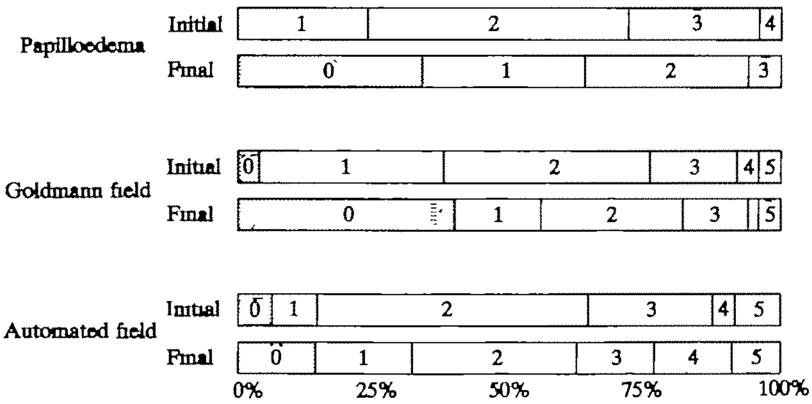

Fig. 5 Papilloedema grade, Goldmann visual field grade and automated visual field grade at the initial and final visits by percentage (grade in worst eye used, normal findings are represented by grade 0 (stippled area)).

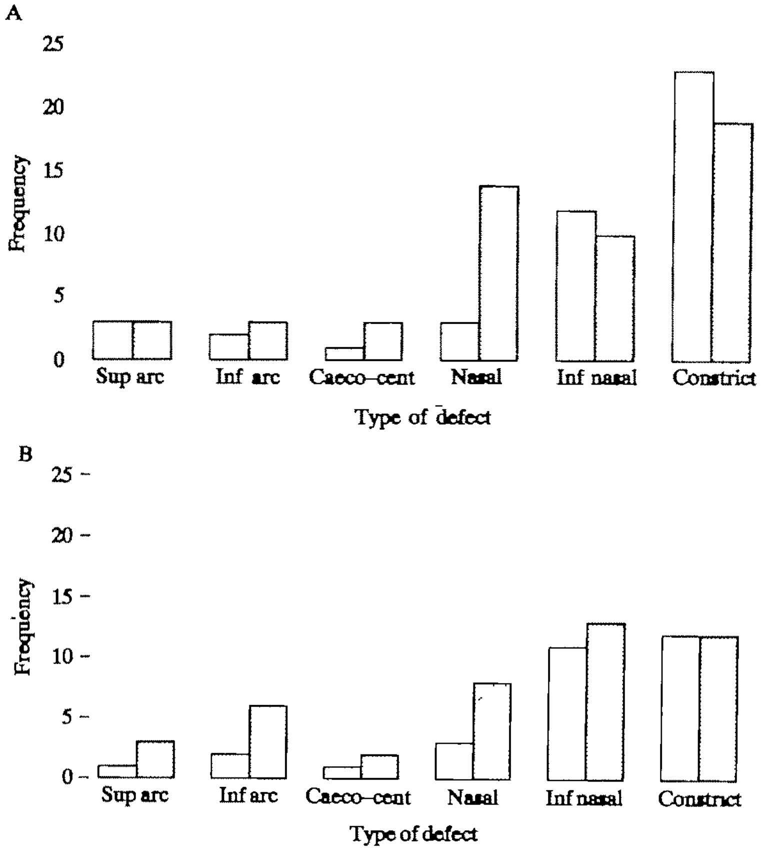

Fig. 6. Goldmann (open areas) perimetry compared with automated (stippled areas) perimetry at the initial (A) and final (B) examinations. Sup arc = superior arcuate scotoma, Inf arc = inferior arcuate scotoma, Caeco-cent = caecocentral scotoma, Inf nasal = inferior nasal defect; Constrict = visual field constriction

automated perimetry there was also a significant improvement in visual field grade from the initial to the final visit ($P = 0.04$); 50% of patients improved and 22% worsened. Four patients with normal Goldmann or automated perimetry at the initial examination (except for blind spot enlargement) later developed visual field defects (3 progressed to grade 1, 1 to grade 2). There was no significant correlation between automated or Goldmann visual field grade and papilloedema grade.

Nine patients had relative afferent pupillary defects; none of the defects resolved. Six patients had sixth nerve palsies; all resolved.

Fig. 5 (top) shows the frequencies in the worst eye of the papilloedema grades of patients at the initial and final visits. There was a significant change in grade from the initial to final examinations ($P < 0.0001$). However, as noted above, papilloedema grade did not correlate with the severity of loss of specific types of visual function (Snellen acuity, contrast sensitivity, visual fields, colour plate testing).

The average optic cup size ratio (vertical cup diameter: total vertical disc diameter) was 0.17 and 0.156 in the right and left eyes, respectively, at the initial visit. The measurement was difficult to estimate in eyes with elevated discs. At the final visit, average cup ratios were 0.20 in both eyes. The change from initial to final examination was significant in the left eye ($P = 0.004$) but not in the right ($P = 0.07$). There was no correlation of final cup size with the presence of loss of visual field by Goldmann perimetry in either eye at the initial or final visit.

Twelve patients (24%) had choroidal folds of which 9 were circumpapillary and 3 radial, 3 had optic atrophy and 1 had optociliary collateral vessels.

Patients with deterioration of visual function

In spite of treatment, visual function deteriorated in 5 patients; i.e., their Goldmann visual field grade was worse at their final examination than initially. Two of these patients were treated with acetazolamide and 3 with acetazolamide and corticosteroids. One of the latter underwent an optic nerve sheath fenestration and worsened with surgery.

To determine the factors related to deterioration, these 5 patients were analysed separately. The following two factors were significantly correlated with deterioration: (1) small optic cup size ($P < 0.03$; Mann-Whitney U test); (2) weight gain over the year preceding diagnosis ($P = 0.0003$); independent t test). There was 1 outlier (45 kg weight gain). If the value of the average weight gain of the others (21 kg) was used for the outlier, the association was still statistically significant ($P = 0.0004$). Of the 5 patients who deteriorated, 1 was treated with corticosteroids for 2 wks before her first visit. She reported a 4.5 kg weight gain over the previous 2 months.

The following factors were not statistically different between the group that deteriorated and the other 45 patients: age, race, sex, obesity, current weight, presence of systemic arterial hypertension, diplopia, intracranial noises, transient visual obscurations, sparkles, retro-orbital pain, retro-orbital pain on eye movement and history of visual loss. In addition, the presence of contrast sensitivity loss, choroidal folds, optic disc atrophy, Snellen acuity loss or colour plate abnormality was not associated with the group that deteriorated. When the Bonferroni adjustment for multiple tests in a single study is applied, weight gain over a 1 yr period before diagnosis remains highly significant even with the outlier correction ($P = 0.006$ and $P = 0.008$).

Treatment

The treatments used and their outcomes are found in Tables 4 and 5. Our number of patients was too small for meaningful statistical analysis.

TABLE 4. TREATMENT OF PATIENTS WITH IIH WITH CORTICOSTEROIDS*

	Papilloedema grade			*Goldmann field grade*			
Case	*Initial*	*End of treatment*	*Final*	*Initial*	*End of treatment*	*Final*	*Months of follow-up after medical treatment*
1	4/4	1/1	–	1/4	0/3	–	2
2	1/1	1/1	1/1	2/2	1/2	1/2	10
3	3/1	0/0	0/0	3/5	3/5	3/5	24
4	1/1	0/0	0/0	2/2	1/2	2/2	2
5	3/3	3/3	Surg.	5/5	5/5	Surg.	Surg.
6	x/2	x/2	Surg.	x/2	x/3	Surg.	Surg.
7	2/3	1/3	0/1	1/1	1/1	0/0	11
8	2/2	2/2	Surg	3/0	3/3	Surg.	Surg.
9	1A/1A	1A/1A	Surg.	4/4	4/4	Surg	Surg.

* Right eye/left eye; A = optic atrophy; x = eye enucleated for melanoma

TABLE 5 CLINICAL COURSE OF TREATED PATIENTS WITH IIH BASED ON GOLDMANN VISUAL GRADE*

	Outcome		
Treatment	*Better*	*Same*	*Worse*
Acetazolamide	11	11	7
Frusemide	9	5	3
Corticosteroids	5	0	4
Surgery	3	2	1

* Three patients were not treated; some patients were treated with more than one medication; 1 patient worsened on corticosteroids and with surgery.

DISCUSSION

In this prospective study we found the incidence of visual field loss to be greater than 90%. We used visual field strategies that tested for the types of defects known to occur with IIH. This contrasts with previous retrospective series that reported much lower rates of visual field loss (Tables 6, 7). We also found a higher incidence of many IIH symptoms.

Symptoms

The most common visual symptoms reported in the cumulated prior series of a total of 1020 patients with IIH were blurring of vision (29%) and diplopia (23%). Transient visual obscurations (20%) and the infrequently mentioned intracranial noises are probably underreported due to the retrospective nature of these studies. Tables 6 and 7 give a comparison of previous reports with the prospectively collected data of this study Tables 1, 3).

TABLE 6. SUMMARY OF PREVIOUS REPORTS OF VISUAL LOSS ASSOCIATED WITH IIH (1020 CASES REVIEWED)

Study	*No. of patients**	*Constriction*	*Nasal loss*	*Central loss*	*Blindness*	*Visual field loss***	*Acuity loss*
Cases with perimetry							
Round and Keane (1988)	101	7	5	?	?	13	16
Sørensen *et al* (1988)	24	2	1	1	1	4	1
Smith and Baker (1986)	22	19	10	11	4	17	10
Orcutt *et al* (1984)	68	50	12	13	7	33	?
Wall *et al.* (1983)	45	9	7	6	0	12	4
Corbett *et al.* (1982)	57	15	18	10	14	20	4
Rush (1980)	63	11	7	5	1	18	7
Bulens (1979)	20	1	2	2	2	4	6
Total	400	114	62	48	29	121	48
Percentage		29	16	12	7	30	12
Perimetry not performed on all cases							
Vassilouthis and Uttley (1979)	28	0	1	5	2	6	7
Weisberg (1975)	120	?	2	?	1	2	6
Johnston and Paterson (1974)	110	?	?	+	?	16	50
Boddie *et al.* (1974)	34	3	1	?	?	4	4
Guidetti *et al.* (1968)	81	+	?	?	?	12	?
Rabinowicz *et al.* (1968)	8	1	2	?	?	3	0
Lysak and Svien (1966)	46	2	4	?	?	6	13
Wilson and Gardner (1966)	48	+	77	?	?	9	13
Paterson *et al* (1961)	17	?	?	?	?	?	?
Dunn *et al* (1955)	4	1	2	?	?	2	3
Foley (1955)	60	5	?	6	3	6	?
Giller and Cogan (1952)	7	2	2	?	1	3	2
Cross (1948)	20	1	1	4	?	4	?
Dandy (1937)	22	1	?	?	1	5	6
Davidoff and Dyke (1937)	15	2	?	?	1	?	10
Total	620	18	22	15	7	78	114
Percentage		3+	4	2	1	13	18
Both sections of table (%)		13	8	6	3	20	16

* No. of patients in study. ** Does not include blind spot enlargement

In the cumulated series, 29% of patients reported visual loss. In the series of Corbett *et al.* (1982), 9 patients (16%) reported visual loss; 7 (12%) became blind in one or both eyes. In our series, 4% of patients became blind (Goldmann field grade 5) in both eyes. Visual loss was noted by 1 in 3 patients, whereas 94% of patients had visual loss by Goldmann perimetry. The low incidence of the symptom of visual loss is undoubtedly due to the insidious deterioration of vision that often spares central vision until late in the course of the illness. The frequency of subclinical visual loss underscores the need for thorough ophthalmological examination with perimetry using a disease specific strategy for patients with IIH.

Most patients in our study sought medical attention for headache or transient or sustained visual loss but 2 asymptomatic patients were found to have papilloedema on routine ophthamological examination.

Headache. The presence of headache is ubiquitous in patients with IIH and is the usual presenting symptom. In this series, most patients with IIH had severe daily

TABLE 7 SUMMARY OF PREVIOUS REPORTS OF OTHER VISUAL SYMPTOMS AND SIGNS ASSOCIATED WITH IIH (1020 CASES REVIEWED)

Authors	*TVO*	*Blurring*	*Diplopia*	*VI palsy*	*Atrophy*
Round and Keane (1988)	53	21	26	14	?
Sørensen *et al.* (1988)	18	5	4	4	1
Smith and Baker (1986)	8	4	3	2	5
Orcutt *et al.* (1984)	37	20	15	15	4
Wall *et al.* (1983)	6	4	1	1	1
Corbett *et al.* (1982)	41	17	19	19	?
Rush (1980)	29	43	22	10	5
Bulens (1979)	2	11	17	13	2
Vassilouthis and Uttley (1979)	?	17	9	6	?
Weisberg (1975)	6	30	20	24	3
Johnston and Paterson (1974)	?	63	35	26	5
Boddie *et al.* (1974)	6	13	5	3	8
Guidetti *et al.* (1968)	?	?	?	0	?
Rabinowicz *et al.* (1968)	?	3	1	0	3
Lysak and Svien (1966)	?	?	?	?	?
Wilson and Gardner (1966)	?	+	?	8	?
Paterson *et al.* (1961)	?	17	4	4	?
Dunn *et al.* (1955)	?	1	2	2	4
Foley (1955)	?	23	23	20	5
Giller and Cogan (1952)	?	6	2	1	1
Cross (1948)	?	?	?	5	1
Dandy (1937)	?	3	7	?	22
Davidoff and Dyke (1937)	2	6	3	1	?
Percentage of total	20%	29%	23%	19%	7%

TVO = transient visual obscurations.

headaches described as pulsatile. They were different from any previous headache and usually lasted hours. Although uncommon, the presence of radicular pain, possibly from dilatation of spinal nerve root sleeves, may aid in the recognition of this headache syndrome. Experimentally-induced increased ICP in humans provokes variable headache responses (Fay, 1940). Johnston and Patterson (1974) observed no clear relationship between changes in intracranial pressure and clinical symptoms. Thus the mechanism of headache in IIH remains unclear.

Transient visual obscurations. Visual obscurations are transient episodes of blurred vision that usually last less than 30 s and are followed by restoration of baseline visual function. Transient visual obscurations occurred in 72% of our patients. This figure is the same as the frequency of 72% in the series of Corbett *et al.* (1982). In the report of Round and Keane (1988) 53% had obscurations. This symptom was reported in 20% of the prior cumulated retrospective series.

The cause of these episodes is thought by some to be transient ischaemia of the anterior optic nerve or disc (Cogan, 1961). They are not correlated with the degree of intracranial hypertension or with the extent of disc oedema (Hayreh, 1977*b*). Sadun *et al.* (1984), citing their existence in optic nerve drusen, Fuchs coloboma, vitritis and compressive optic neuropathy, proposed their aetiology was transient ischaemia of the optic nerve head due to increased tissue pressure.

Although this symptom has been used by some as an indication for surgical decompression or shunting (Holmes, 1937; Smith, 1958); other authors do not believe that it is associated with a poor visual outcome (Cogan, 1961; Bulens *et al.*, 1979; Rush, 1980; Corbett *et al.*, 1982; Wall *et al.*, 1983). Orcutt *et al.* (1984) have found a significant association between transient visual obscurations and visual loss. They found 88% of eyes with severe visual loss had transient visual obscurations, while this symptom was present in 50% of all eyes. This difference was significant at the 5% level. We found no significant relationship between the symptom of visual obscurations and visual field loss or deterioration of Goldmann visual field grade.

Intracranial noises. Intracranial noises (pulsatile tinnitus) are not mentioned in most reports of patients with IIH. Foley (1955) noted their presence in 11 of 31 cases. They were common in our patients (60%). Although the sound was often unilateral, neither side predominated. Rather than the high-pitched ringing sound characteristic of typical tinnitus, patients described a pulsatile bruit-like sound. In patients with intracranial hypertension, Meador and Swift (1984) reported that jugular compression ipsilateral to the sound abolishes it. Some of our patients also noted this phenomenon. Sismanis (1987) found that the sound disappeared immediately, although temporarily, after lowering CSF pressure by lumbar puncture.

In a prospective study, Sismanis (1987) found pulsatile tinnitus in each of 20 patients with IIH. He found it to be synchronous with the heartbeat. Predominantly low frequency hearing loss occurred in 18 of the 20 patients; it is improved with therapy. Similar temporary improvement occurred with digital pressure over the ipsilateral jugular vein. Auditory brainstem evoked responses were abnormal in 2 of the patients. He attributed the noise to transmission of intensified vascular pulsations via CSF under high pressure to the walls of the venous sinuses. The periodic compressions were thought to convert the laminar to turbulent blood flow. He ascribed the hearing loss to the masking effect of the low frequency hum (Sismanis, 1987).

Ophthalmological abnormalities

Snellen visual acuity. It is generally accepted that visual acuity remains normal in patients with papilloedema except when the condition is of long-standing (Holmes, 1937; Guidetti *et al.*, 1968; Huber, 1976). In the cumulated series (Table 6), 16% of patients had Snellen acuity loss. However, it was not usually stated whether reported acuities were obtained with the best possible optical corrections, and many use 20/30 as the upper limit of normal. At the initial visit, 13% of eyes in our series had acuity worse than 20/20; 10% were abnormal at the final visit. There was no significant change in Snellen acuity from the initial to the final visits. Snellen acuity testing was insensitive to visual loss found by perimetry and to change in perimetry grade. In addition, worsening of papilloedema grade was not associated with change in Snellen acuity.

Contrast sensitivity. Using the Arden gratings to test contrast sensitivity, we have previously found deficits in 54% of eyes. Snellen acuity was worse than 20/20 in 21%. Whereas there was no significant association of change in initial and final acuities with papilloedema grade, there was an association with change in contrast sensitivity scores (Wall, 1986). Verplanck *et al.* (1988) have reported a similar superiority of detection of visual loss by contrast sensitivity testing compared with Snellen acuity. They found predominantly middle and high spatial frequency loss. Bulens *et al.* (1988) have also noted this type of loss of contrast sensitivity with 8 of 37 eyes having a middle spatial frequency 'notch' loss and 7 having high spatial frequency loss. Our results are similar to these studies. Contrast sensitivity is more sensitive in detecting change than is Snellen acuity. In addition, in our study contrast sensitivity was the only visual parameter that correlated significantly with the symptom of sustained visual loss.

Colour vision. Colour plate loss occurred in 20% of patients at their initial visit. Colour plate tests were developed to separate congenitally colour deficient subjects from the rest of the population. They are generally insensitive for the detection of acquired dyschromatopsia (Wall and Dalali, 1989). We have found a much greater incidence of abnormality in patients with IIH with the Farnsworth-Munsell 100 hue test and the Lanthony desaturated 15 hue test (unpublished data).

Ophthalmoscopic examination. We did not attempt to correlate the degree of visual loss with ICP. Since pressure fluctuates throughout the day (Johnston and Paterson, 1974) long-term ICP monitoring would be required. Instead we used papilloedema grade.

Smith and Baker (1986) reported that severe papilloedema was associated with visual loss, whereas Rush (1980) has stated there is no relationship. Orcutt *et al.* (1984) found a significant correlation between high grade papilloedema and atrophic papilloedema and visual loss. Our findings confirm the observation of Corbett *et al.* (1988*b*) that in the individual patient, the severity of visual loss cannot be predicted from the severity of the papilloedema. Partial explanation for this is with axonal death from compression of the optic nerve head, the amount of papilloedema decreases.

Beck *et al.* (1984) have noted the association of small or absent optic cups in patients with disc oedema from ischaemic optic neuropathy. Orcutt *et al.* (1984) have suggested that a small scleral canal in IIH patients may increase the vulnerability of axons to subsequent damage. James Corbett (personal communication) has found in an analysis of his data that there is a tendency for IIH patients to have a small optic cup. We found an association between patients with deterioration of Goldmann visual field grade and a small optic cup but it was not significant after correcting for multiple statistical tests. However, we also found no association between small cup size at the final visit and high automated or Goldmann visual field grade.

Choroidal folds are known to occur in IIH (Bird and Sanders, 1973). Corbett (personal communication) has noted the presence of circumpapillary choroidal folds to be a predictor of visual loss in IIH. We were unable to confirm this observation.

Pupil examination. Although the presence of a relative afferent pupillary defect is a sensitive indicator of the presence of a unilateral optic neuropathy, it is often absent in IIH. This is because its existence depends on asymmetry of visual loss and the optic neuropathy of IIH is usually symmetric. In this series, relative afferent pupillary defects were present in 18% of patients. Corbett *et al.* (1982) has noted the finding in 28% of patients and Digre and Corbett (1988) in 34% of men with IIH.

Perimetry. We found visual loss in at least one eye in 96% of patients with Goldmann perimetry and in 92% with automated perimetry. In a retrospective study that used a nonspecific two isopter ($I4_e$, $I2_e$) screening strategy, one-quarter of patients had visual loss (Wall *et al.*, 1983). Others have found rates of loss of 13–49% but the visual field testing stategy was not mentioned (*see* Table 6; Bulens *et al.*, 1979; Rush, 1980; Corbett *et al.*, 1982; Orcutt *et al.*, 1984; Round and Keane, 1988; Sørensen *et al.*, 1988). Investigators using automated perimetry of the central 30° have reported 3 of 4 eyes of patients with IIH have defects (Smith and Baker, 1986; Wall and George, 1987).

The visual field defects found in IIH are the same as those reported to occur in papilloedema due to other causes. These 'disc-related defects' are the same as those found in glaucoma (Grehn *et al.*, 1981). These defects are enlargement of the blind spot (Knapp,1870), constriction of isopters, loss of inferonasal portions of the visual field (Dersh and Schlezinger, 1959), central, paracentral and caecocentral scotomas (Grehn *et al.*, 1981; Wall and George, 1987), altitudinal patterns of loss (Rush, 1980) and arcuate scotomas (Cross, 1948). The loss of visual field may be progressive and severe, leading to total blindness (Foley, 1955; Corbett *et al.*, 1982).

Blind spot enlargement is ubiquitous in IIH. Since refraction often eliminates this defect (Corbett *et al.*, 1988*a*), we do not consider blind spot enlargement significant visual loss unless it encroaches on fixation. We have therefore focused on other defects.

The most common types of defects reported have been constriction and loss of inferonasal portions of the visual field. Our findings (Table 3, fig. 6) agree with these previous reports. Arcuate scotomas have been reported in association with papilloedema (Löhlein, 1914) and IIH (Cross, 1948). This was another common defect observed in our series; it has not been noted in many other series. Altitudinal defects were uncommon and temporal sector defects were notably rare. Central or paracentral scotomas were reported in 12% of patients in the previous studies in which perimetry was performed. In our patients, 6% had central or paracentral defects with Goldmann perimetry and 14% with automated perimetry.

Comparing the manual (Goldmann) and automated perimetry strategies, manual perimetry was superior at defining step defects along the nasal horizontal meridian (Table 3, fig. 6). Generalized loss on the nasal side of the visual field was common with the automated strategy, even when Goldmann perimetry showed only an inferonasal sector defect. This statokinetic dissociation (Wedemeyer *et al.*, 1989) revealed a circumstance where the automated strategy was superior. Detection of shallow scotomas, especially in the Bjerrum area was also greatest with automated threshold static perimetry.

The only other study that has collected data on visual testing in a systematic prospective fashion is that of Sørensen *et al.* (1988). They followed 24 patients with Snellen acuity and tangent screen visual field examination. However, their tangent screen strategy used a 3 mm white target at 1 m and a 16 mm target at 3 m. With these intense targets it was not surprising that only 18% of their patients had visual field defects.

In our study, there was significant improvement in perimetry grade from the initial to the final examination: 60% of patients had improvement in Goldmann visual field grade and 50% had improvement of automated visual field grade (fig. 5). The reversibility of visual field loss in IIH is not widely appreciated.

Although most patients' vision improved, 5 had worsening of their Goldmann visual field grade. Recent weight gain was the only factor significantly associated with this change. Besides recent weight gain, another group at risk for severe visual loss of which we had 1 patient with marked visual loss, is the black male (Digre and Corbett, 1988).

Recommendations for visual testing. Our data confirm our earlier report that Goldmann perimetry with Armaly-Drance strategy or automated perimetry of the central 30° are sensitive routines for detection of visual loss in patients with IIH (Wall and George, 1987). Automated perimetry of the area outside 30° is often unreliable, time consuming, difficult for the patient and has high test-retest variability. After reviewing our first 17 patients using the full field strategy on the initial visit, we concluded it less useful than the program thresholding test loci within the central 30°. Contrast sensitivity loss is also a sensitive indicator of loss of vision in patients with IIH. Snellen acuity and confrontation visual fields are insensitive tests and should not be relied upon to determine whether visual loss is present. We did not use the visual evoked response to evaluate our patients. Verplanck *et al.* (1988), using check sizes of 15, 30 and 60 min in patients with IIH, found the visual evoked response insensitive to the presence of visual loss when compared with perimetry or contrast sensitivity.

The results of this study emphasize that patients with IIH should be evaluated with perimetry with a known sensitive strategy and contrast sensitivity testing. Automated perimetry is used for attentive and motivated subjects. Unfortunately, because of the variability inherent in the test, the determination of early change in the visual field is problematic. These psychophysical tests should be correlated with indirect ophthalmoscopy and stereoscopic optic disc photographs if available (Corbett and Thompson, 1989).

Mechanisms of visual loss

The proposed pathophysiological mechanisms for visual field loss in IIH are damage to the retina, optic disc and optic nerve.

In a study of experimental papilloedema in monkeys, Tso and Hayreh (1977*a, b*) in the retrolaminar optic nerve found only axonal degeneration with collapse of the myelin sheath and loss of axoplasm. The prelaminar portion of the nerve (the unmyelinated portions of the fibres within the disc itself) showed severe axonal swelling and mild interstitial oedema. In a series of papers (Hayreh, 1977*a, b*; Hayreh and Hayreh, 1977*a, b*; Tso and Hayreh, 1977*a, b*) the authors reported findings to support the hypothesis that optic nerve damage in chronic papilloedema is the result of ischaemic damage to the optic disc.

We agree that the anterior optic nerve is the site of the lesion responsible for visual field defects in IIH. The evidence for this is as follows. (1) The same types of visual field defects occur in other diseases known to affect the optic nerve head. The most common of these are glaucoma (Armaly, 1969; Aulhorn and Karmeyer, 1977) ischaemic optic neuropathy (Boghen and Glaser, 1975; Hayreh, 1975; Aulhorn and Tanzil, 1979), and so-called low tension glaucoma (Levene, 1980; Motolko *et al.*, 1982). (2) Damage to the chiasmal and retrochiasmal visual system is manifest in visual field defects that have borders that respect the vertical midline. None of our 234 examinations showed this type of defect. (3) There is circulatory stasis in the prelaminar optic nerve associated with disc oedema from increased intracranial pressure (Paton and Holmes, 1911; Rios-Montenegro *et al.*, 1973; Hayreh and Hayreh, 1977*a*). With papilloedema there is delay in the slow phase of axoplasmic transport in all fibres (Minckler *et al.*, 1976; Parhad *et al.*, 1982) and in rapid axoplasmic transport in some fibres (Radius and Anderson, 1980). (4) Although the severity of visual loss in the individual patient cannot be predicted from the severity of papilloedema, there appears to be more visual loss in groups of patients with high grades of papilloedema. In addition, Orcutt *et al.* (1984) found a significant correlation between high grade papilloedema and atrophic papilloedema and visual loss. Smith and Baker (1986) have found a higher incidence of abnormal visual fields with higher grades of papilloedema.

Whether these lesions, of the anterior optic nerve, produce visual defects by ischaemia alone, by infarction or by some other mechanism is unclear. Another possibility is that conduction defects are related to the stasis of axoplasmic flow found in papilloedema (Tso and Hayreh, 1977*b*). This mechanism might explain the reversibility of some of the defects. It is also compatible with gradual recovery of visual function following surgical intervention. Since the greatest density of axons is in the temporal arcuate bundles, a speculation is that with flow stasis and resultant compression, defects would appear in these areas more often (thus the greater frequency of nasal as opposed to temporal defects).

Another mechanism to account for central scotomas is involvement of the macula. There may be extension of oedema from the disc into the macula (Dunn *et al.*, 1955). In addition macular haemorrhages, macular pigment epithelial changes and neovascular membrane formation have been reported in cases of papilloedema (Morris and Sanders, 1980; Gittinger and Asdourian, 1989).

Enlargement of the blind spot is due primarily to the peripapillary retina being hyperopic (Corbett *et al.*, 1988*a*). Elevation and detachment of peripapillary retina (Samuels, 1938) and choroidal folds (Frisén and Holm, 1977) have also been proposed to produce defects contiguous with the blind spot. It appears that defects from choroidal folds result from an angular misalignment of photoreceptors (Stiles-Crawford effect).

Treatment

Subtemporal or suboccipital decompression was used from the 1940s to the 1960s for patients with threatened visual loss from IIH. While some patients showed improvement, others were unrelieved, and some went blind (Dandy, 1937; Bradshaw, 1956; Bulens *et al.*, 1979). These procedures have been abandoned because of their many complications.

The role of weight loss in the treatment of symptomatic patients with IIH is unclear. Newborg (1974) reported remission in all 9 patients placed on a 400 to 1000 calorie rice diet. There was no mention of the patients' visual testing. Greer (1965), however, reported a group of 6 obese patients that became asymptomatic without weight loss. Amaral *et al.* (1987) noted resolution of IIH in a patient following surgically induced weight loss (gastric exclusion procedure). Since recent weight gain was strongly associated with visual deterioration in the present study, we recommend weight loss as a treatment for IIH.

Repeated lumbar punctures have been used to treat IIH. Weisberg (1975) reported 6 of 28 patients treated with serial lumbar punctures as symptomatically improved. Use of this treatment is tempered by the observation that CSF is replenished in 1–2 h if no CSF leak occurs (Johnston and Paterson, 1974) and some patients will risk visual loss rather than return for serial lumbar punctures.

The most common medications used to treat IIH are diuretics and corticosteroids. Paterson *et al.* (1961) first reported the efficacy of corticosteroids for treating IIH. Other studies have also suggested that corticosteroids are effective, but these were neither randomized nor controlled (Weisberg, 1975; Weisberg and Chutorian, 1977). Although most patients in our series that were treated with steroids also responded, there was recurrence in some with rapid tapering of the dose. A prolonged tapering may prevent the return of symptoms in some cases.

Acetazolamide, a diuretic that also reduces CSF production, was reported to be effective by Lubow and Kuhr (1976). Gücer and Viernstein (1978), using ICP monitoring, showed acetazolamide to be effective in lowering ICP in doses of 2–4 g/day. Tomsak *et al.* (1988) have documented resolution of papilloedema with photographs of the optic disc in patients treated with 1 g/day of acetazolamide. Acetazolamide appeared to be an effective medication in our uncontrolled study.

Frusemide has also been used to treat IIH (Corbett, 1983). Nine of our patients responded favourably to frusemide with improvement of papilloedema or visual field; 5 others had no change in their visual testing and 3 worsened. As with all treatments of IIH, all reports to date are anecdotal and recommended treatments vary widely. A randomized controlled study is needed to determine the efficacy of the medical forms of therapy.

The surgical forms of therapy now used are various shunting and decompression procedures: lumbar subarachnoid-peritoneal shunt and optic nerve sheath fenestration. Lumbar subarachnoid-peritoneal shunts (Vander Ark *et al.*, 1971) may arrest or reverse loss of vision. However, because of high rates of shunt failure and other complications (Rosenberg *et al.*, 1989), the procedure is falling into disfavour. Optic nerve sheath fenestration is an effective method for reversing visual loss and protecting the optic nerve from further damage (Galbraith and Sullivan, 1973; Sergott *et al.*, 1988). However, patients can lose vision in the perioperative period (Corbett *et al.*, 1988*b*).

We treated our patients with an algorithm that used change in visual function to determine the type of intervention (Wall and Weisberg, 1985). Since in individual patients symptoms and the degree of papilloedema may not correlate with loss of visual function, we used the results of Goldmann perimetry to determine treatment. If the visual loss was mild, acetazolamide or frusemide was used. If the visual loss progressed or the visual loss was initially moderate or severe, optic nerve sheath fenestration or a 3 month course of corticosteroids was used. If visual loss progressed in spite of steroids or if it returned during a slow steroid tapering, an optic nerve sheath fenestration was then performed. If following this, headache was severe and did not respond to medication other than steroids, a lumbar-subarachnoid peritoneal shunt was performed. We did not use serial lumbar punctures.

Study weaknesses or deficiencies. A weakness of the study is that our series is too limited and uncontrolled to draw conclusions about the efficacy of therapy. Another weakness is the wide variation in the length of follow-up. Ideally, the patients should have been followed at systematic intervals and some for a longer time period.

Conclusions

In this prospective study, the results of our five study outcomes, as proposed in the Methods section, are as follows. (1) With treatment, both papilloedema grade and perimetry grade improve; however, (2) the severity of the papilloedema does not correlate with perimetry grade; (3) the presence of transient visual obscurations does not correlate significantly with visual loss on the initial examination as determined by Goldmann visual field grade; (4) the final optic cup size does not correlate with visual loss by Goldmann perimetry; and (5) of the many factors tested, only weight gain over the year preceding diagnosis is significantly associated with deterioration of vision as determined by Goldmann visual field grade.

The results of both Goldmann and threshold automated perimetry were abnormal in over 90% of the patients in this study. About half of these patients had defects in the visual field periphery that were unlikely to compromise daily activities. However, this visual loss serves as a marker for guiding treatment.

Although the mechanisms of IIH remain obscure, it has become clear that loss of visual function is common and patients may progress to blindness. Hence the term 'benign intracranial hypertension' should be discarded. Patients with IIH should be tested serially with several sensitive visual function tests, including perimetry using an appropriate strategy. This should be undertaken so the proper therapy can be selected and visual loss prevented or reversed.

ACKNOWLEDGEMENTS

The authors thank Drs Leon Weisberg and James Corbett for their helpful comments and Dr Paul Siegel for editing and epidemiological insights. Dr Janet Hughes assisted with the statistical analyses

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(Received August 8, 1989. Revised December 19, 1989. Accepted January 24, 1990)

APPENDIX

Visual field grading criteria for manual and automated perimetry

I. *Goldmann perimetry*

- A. Grade 0. Normal visual field
- B. Grade 1. Minimal visual loss (unlikely to be noticed by the patient)
  - 1. Isopter constriction
    - step defects present that are less than 10° but greater than 5° in diameter
  - 2. Defects not involving fixation
    - (a) relative scotomas that do not involve fixation
      - (1) up to 20°×20° in area outside 30°
      - (2) up to 10° in area inside 30°
    - (b) blind spot enlargement—involves central 10° but not fixation
  - 3. Defects involving fixation: none—acuity is 20/20 or better
- C. Grade 2. Mild visual field loss (may be noticed by patient—usually compromises function)
  - 1. Isopter constriction
    - (a) up to 20° in area
    - (b) $I4_e$ isopter inside 30° nasally, 50° temporally
    - (c) $I2_e$ isopter inside 20°
  - 2. Defects not involving fixation
    - (a) relative: less than one quadrant in size, defect greater than criteria of I.B.2.a
    - (b) absolute: less than 20°×20° in area
  - 3. Defects involving fixation: acuity 20/30 or better
- D. Grade 3. Moderate visual field loss (nearly always noticed by patient—interferes with function)
  - 1. Isopter constriction
    - (a) greater than 20° to any isopter but more than 50° of field to the $V4_e$ stimulus
    - (b) $I3_e$ isopter inside blind spot
    - (c) $I2_e$ isopter inside 10°
  - 2. Defects not involving fixation
    - (a) relative: greater than one quadrant but less than one hemifield
    - (b) absolute: greater than 20°×20° in diameter but less than one quadrant
  - 3. Defects involving fixation—acuity greater than 20/30 but less than 20/100
- E. Grade 4. Marked visual field loss
  - 1. Constriction—less than 50° but greater than 20° in diameter to $V4_e$
  - 2. Defects not involving fixation
    - (a) relative: one hemifield or greater with more than 20° of field left to $V4_e$ stimulus
    - (b) absolute: greater than one quadrant with more than 20° to $V4_e$ stimulus
  - 3. Defects involving fixation—acuity 20/100 to 20/200
- F. Grade 5. Blinding visual field loss
  - 1. Constriction—less than 20° to $V4_e$
  - 2. Acuity worse than 20/200

II. *Automated perimetry*

- A. Grade 0. Normal visual field
- B. Grade 1. Minimal visual loss
  - 1. Meets criteria for deficit but no greater (3 contiguous points abnormal, no point with loss greater than 10 dB)
  - 2. Enlarged blind spot defined by presence of 5 or more disturbed points adjacent to the blind spot with no encroachment into the central 10°
  - 3. Defects involving fixation—none, acuity 20/20 or better
- C. Grade 2. Mild visual field loss
  - 1. Defect greater than criteria for deficit (B.1.)
  - 2. Enlarged blind spot defined by presence of 5 or more disturbed points adjacent to the blind spot with encroachment into the central 10°
  - 3. Defects involving fixation—less than 10 dB loss; acuity 20/20 or better
- D. Grade 3. Moderate visual field loss
  - 1. Isopter constriction—all points abnormal in one isopter
  - 2. Defects not involving fixation
    - (a) relative: greater than one quadrant (all points involved) but less than one hemifield
    - (b) absolute: greater than 20°×20° in diameter but less than one quadrant

   3. Defects involving fixation—acuity worse than 20/30 but less than 20/100, blind spot encroaching on fixation (relative defect) greater than 10 db loss
- E. Grade 4. Marked visual field loss
   1. Constriction—less than 50° but more than 20° to brightest stimulus
   2. Defects not involving fixation
      - (a) relative. one hemifield or greater with more than 20° of field left to brightest stimulus
      - (b) absolute: greater than one quadrant with more than 20° to brightest stimulus
   3. Defects involving fixation
      - (a) acuity worse than 20/100 to 20/200
      - (b) blind spot encroaching on fixation (relative defect) greater than 2 log units of loss
- F. Grade 5 Blinding visual field loss
   1. Constriction—less than 20° to brightest stimulus
   2. Acuity worse than 20/200 or greater than 3 log unit loss at fixation

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INTRATHECAL IMMUNOGLOBULIN M SYNTHESIS IN MULTIPLE SCLEROSIS

RELATIONSHIP WITH CLINICAL AND CEREBROSPINAL FLUID PARAMETERS

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SUMMARY

Intrathecal IgM synthesis was assessed through the detection of oligoclonal bands in cerebrospinal fluid (CSF) and through calculation of IgM index values and total CSF IgM amounts. Intrathecal production of IgM was then evaluated along with other CSF parameters and clinical variables in 150 patients with multiple sclerosis (MS). Intrathecal production of IgM was detected in 55% of patients and was found to correlate with disease activity, manifesting as a recent relapse, as well as the total number of relapses. An inverse correlation was found between CSF oligoclonal IgM and both the time interval between the last relapse and lumbar puncture, and duration of the disease process. It also correlated with the number of leucocytes, free kappa and lambda light chain bands in CSF. No correlation was found between intrathecal IgM synthesis and degree of disability (Kurtzke scale), disease progression index (Poser scale), or total cerebral plaque burden (detected by enhanced CT and MRI scans). Oligoclonal IgM bands were found to be more specific for active disease process than either IgM index or total CSF IgM amount. It is concluded that intrathecal synthesis of IgM, as detected by CSF oligoclonal bands, is a useful parameter in monitoring disease relapse activity in MS.

INTRODUCTION

The most consistent abnormality detectable in cerebrospinal fluid (CSF) from patients with multiple sclerosis (MS) is the increased intrathecal humoral immune response, which is usually detected in the form of oligoclonal bands (OCB) observed in electrophoresed, or electrofocused, CSF. These discrete bands, which have restricted charge characteristics that differ from immunoglobulins in homologous serum, represent the immunological response of a limited number of lymphocyte clones within the intrathecal compartment to a restricted number of antigenic determinants. They are, therefore, valuable clinical markers for B-cell proliferation in MS brains.

Most research work on the immunoglobulin (Ig) composition of CSF OCB is aimed at IgG with less emphasis on IgM, mainly because of methodological difficulties involved in its detection. From an immunological standpoint, the detection of alterations in CSF IgM should be more useful than that of IgG because the former is both ontogenetically and phylogenetically the most primitive Ig and is thereby detected at an earlier stage of the immune response. It is the first Ig to return to normal when the offending antigen disappears, and its persistence is regarded as a sign of continuous antigenic stimulation.

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Thus detection of intrathecal IgM production could be useful not only for diagnostic purposes but also to follow the course of the immune response in inflammatory neurological conditions.

Reference values for CSF IgM levels have been previously described (Nerenberg *et al.*, 1978; Sindic *et al.*, 1982; Forsberg *et al.*, 1984; Felgenhauer and Schädlich, 1987; Sharief and Thompson, 1989), and the electrophoretic distribution of IgM in CSF of MS patients has only recently been reported (Keir *et al.*, 1982; Walsh *et al.*, 1985). However, the clinical implications of CSF IgM OCB in patients with MS have not hitherto been studied properly and the important question as to whether the IgM immune response is related to the development of new lesions or to random entry of B-cells into the MS brain has not yet been clearly answered.

This study reports the incidence of IgM OCB in CSF of MS patients, considers this finding in relation to disease duration, disability status, activity (manifested by recent relapse), and progression index (as defined by Poser *et al.*, 1982), and correlates the presence of CSF IgM OCB with that of other CSF parameters. The implications of CSF IgM OCB in MS will also be discussed in terms of disease pathogenesis and diagnosis.

PATIENTS AND METHODS

Patients

Patients with MS included in this study were selected from a group of 560 patients with various neurological disorders who had been admitted for investigation and/or treatment to the National Hospital for Nervous Diseases, Queen Square. All patients with the final diagnosis of MS were selected for the study and lumbar CSF and corresponding serum samples were examined within 1 wk of the clinical evaluation. Detailed history and neurological findings were recorded and all clinical judgements and decisions were taken according to the standards of the National Hospital.

The MS patients were then classified (McDonald and Halliday, 1977) and only those with clinically definite MS and early probable or latent MS were included in the study (Table 1). The whole study was performed blind, and patients who were receiving immunosuppressive therapy within 6 months of the lumbar puncture were excluded.

TABLE 1 SEX AND MEAN AGE OF PATIENTS IN DIFFERENT CLINICAL GROUPS OF MS

	Sex			
Clinical group	*Female*	*Male*	*Total number*	*Mean age (yrs)*
Clinically definite				
Relapsing	21	16	37	31.7
Progressive	10	5	15	36.2
Relapsing and progressive	12	8	20	34 4
Early probable or latent	47	31	78	33.7
Total	90	60	150	33.9

Clinical assessment

Some 80 MS patients were examined and evaluated clinically by one of us (M.K.S.) within a week of the lumbar puncture, and the degree of their disability was assessed and scored according to Kurtzke's

Disability Status Scale (KDSS, Kurtzke, 1965). Clinical data on the remaining 70 patients were gathered through examination of hospital notes where considerable caution was exercised in scoring the degree of disability. Those patients whose hospital notes were judged to be insufficient for complete clinical assessment were excluded from the study.

A variety of parameters were used to assess clinical activity of the disease process. Patients with relapsing-remitting forms of the disease, including those with early probable or latent MS, were studied during acute exacerbations to ensure that they had active disease during the time of lumbar puncture. Other indicators of disease activity include rapidly worsening disability rate, documented occurrence of new symptoms and neurological signs, and development of new CNS lesions as detected either by neuroimaging or consecutive clinical exmainations. Disease activity at the time of the lumbar puncture was also assessed by enhanced CT scans and MRI studies (*see below*). The Progression Index of each patient was calculated according to Poser *et al.* (1982) as the grade on the KDSS divided by the years of disease duration.

Methods

Agarose gel electrophoresis. IgM OCB in unconcentrated CSF was detected by the method of glutaraldehyde-enhanced immunofixation (Sharief *et al.*, 1989) where agarose gel electrophoresis was used for initial separation of CSF and serum proteins before immunostaining for IgM bands. Briefly, CSF and diluted homologous serum specimens containing equal amounts of IgM are electrophoresed then separated proteins are transferred passively to nitrocellulose membrane which is subsequently treated with 1% glutaraldehyde. IgM bands are later detected by peroxidase-conjugated $F(ab')_2$ fragment of antihuman IgM antibody which exclusively stains IgM.

Each set included IgM OCB-positive and IgM OCB-negative control specimens. Corresponding serum should always be examined in parallel to exclude monoclonal or oligoclonal IgM bands that may be present in serum (e.g., in benign monoclonal gammopathy, myeloma, or bacterial meningitis) which transudate into CSF. Such a pattern may be interpreted as local intrathecal synthesis if only CSF is examined.

Determination of IgM content and IgM index. The electrophoretic procedure was standardized for the amount of CSF and serum IgM applied on the gel which implies that IgM content of the test sample should be determined prior to electrophoretic separation. Such determination is carried out by a sandwich enzyme-linked immunosorbent assay (ELISA) where IgM content of the test sample is captured by a polyclonal u-chain specific anti-IgM antibody and then detected by a peroxidase-conjugated $F(ab')_2$ of antihuman IgM antibody (Sharief and Thompson, 1989). The ELISA technique was also used to evaluate intrathecal IgM synthesis through determining total CSF IgM amount and calculating IgM index value (Frydén *et al.*, 1978) which was calculated as follows:

$$\text{IgM Index} = \frac{\text{CSF IgM} \times \text{serum albumin}}{\text{serum IgM} \times \text{CSF albumin}}$$

Other CSF studies. Isoelectric focusing to detect IgG OCB in unconcentrated CSF was performed, on agarose gel, on all specimens according to the method of Walker *et al.* (1983). Again, homologous serum should be examined in parallel with CSF before interpreting the significance of CSF OCB. Detecting CSF free light chain (FLC) bands was by the method of replicate immunoblotting (Thompson and Keir, 1984), where CSF containing 100 μg of total protein is electrophoresed on polyacrylamide gel then separated proteins are transferred to nitrocellulose membranes which are subsequently immunostained for kappa and lambda light chain bands respectively. The CSF white cell count was performed using Fuchs-Rosenthal chambers and cytological examination was performed using Giemsa stain from a Shandon cytocentrifuge preparation (Thompson *et al.*, 1975).

Imaging studies. Magnetic resonance imaging (MRI) was performed on 50 patients using a Picker 0.5 Telsa superconducting MR imager. Axial sections of the brain were made using a spin-echo multislice sequence (SE 2000/60). A pulse repetition rate (TR) of 1.5 s was used with an echo delay time of 80 ms, producing a relatively T_2-weighted scan to detect any pathology. A second sequence was performed with a TR of 0.5 s and an echo delay time of 40 ms, producing a relatively T_1-weighted scan to depict the anatomy. The section thickness was 5 mm. Lesions size, location and degree of atrophic changes were recorded for further evaluation (*see below*).

Almost all patients were assessed by plain and enhanced computerized tomographic (CT) scans. The combination of enhanced CT and MRI scans can provide valuable information about MS lesions (Koopmans *et al.*, 1989). Scans were performed with a General Electric 9800 scanner using 10 mm contiguous slices and were obtained before and 1 h after the i.v. administration of a contrast (usually Isopaque). The presence of low density and enhancing lesions was assessed and the number and location of such lesions were recorded. Evaluation of MRI and CT scan results was performed blind to clinical or pathological findings. The total number of plaque lesions was first counted then the size of each plaque was graded from 1 to 5, with higher numbers indicative of larger size. Lesions less than 1 cm were given a score of 1, 1 cm = 2, 1.5 cm = 3, 2 cm = 4, >2 cm = 5. The location of plaque lesions was categorized as periventricular, subcortical and centrum ovale, corpus callosal, brainstem, and cerebellar.

Statistical methods. Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS). The statistical method of discriminant analysis (Goldstein and Dillon, 1978) was used to determine whether various clinical and laboratory parameters correlate with, or distinguish between, patients who show IgM OCB in their CSF and those who do not. It was also of interest to determine which variables correlate most with CSF IgM OCB.

While descriptive statistics and univariate tests of significance were used in calculations, multivariate statistical procedures, mainly discriminant analysis, were employed first to analyse the variables together, not singly. Because various clinical and laboratory variables in patients with MS could be interrelated, considering them simultaneously will incorporate important information about their relationships with each other and with CSF IgM OCB.

RESULTS

Of the 150 patients, 90 (60%) were female and 60 (40%) male (Table 1). The mean age of onset was 33.9 (SD 8.9, range 14–67) yrs. Patients with relapsing/remitting MS were significantly younger than those with progressive disease ($P < 0.05$). Patients with relatively low disability scores predominate, mainly because most CSF specimens used in this study were obtained from diagnostic lumbar punctures (Table 2).

TABLE 2. DISABILITY SCORES (KDSS) ACCORDING TO CLINICAL GROUP

	Disability score		
Clinical category	*0–3*	*4–5*	*6–7*
Clinically definite			
Relapsing	18	14	5
Progressive	6	6	3
Relapsing and progressive	8	7	5
Early probable or latent	53	21	4
Total	84	49	17

Intrathecal production of IgM was manifested by the presence of IgM bands in CSF that are absent from homologous serum. Caution was exercised in that no negative sample (i.e., a sample with no detectable intrathecal IgM synthesis) was due to an insufficient amount of IgM being applied for electrophoresis. Thus all negative samples demonstrated some IgM reactivity in the form of a diffuse polyclonal pattern. A single IgM band was detected in the CSF of 6 patients but no corresponding band was seen in homologous serum, implying that the CSF band is the result of intrathecal synthesis. Another 8 patients had multiple bands in CSF and only 1 band in homologous serum. Such a pattern was also regarded as indicative of local intrathecal IgM synthesis. All the remaining patients who were positive for IgM OCB demonstrated multiple CSF IgM bands (maximum of 5) with no corresponding banding pattern in homologous serum.

IgM OCB were found in 82 of 150 patients (55%). Of the 72 patients with clinically definite MS, 39 (54%) had CSF IgM OCB and another 55% of patients with early probable or latent MS demonstrated such findings (Table 3). No statistically significant correlation was detected between oligoclonal CSF IgM and any particular clinical group of MS.

TABLE 3 OLIGOCLONAL IgM BANDS AND IgM INDEX VALUES IN DIFFERENT DISEASE CATEGORIES

	Oligoclonal IgM		*IgM index value*	
Clinical group	*Negative*	*Positive*	*≤ 0.068*	*> 0.068*
Clinically definite				
Relapsing	13	24	16	21
Progressive	9	6	8	7
Relapsing and progressive	11	9	10	10
Early probable or latent	35	43	32	46
Total	68	82	66	84

A reference IgM index range was determined by calculating index values for 30 healthy control subjects. The mean index value in the reference population was 0.027 with 95% population limits of 0.01 and 0.068. Index values in different clinical groups of MS are shown in Table 3. Intrathecal IgM production detected by raised IgM index values correlated well with CSF OCB ($P < 0.0001$). Nonetheless, we considered the qualitative detection of CSF IgM OCB as the standard method of demonstrating intrathecal IgM synthesis, since in a separate study we found it more reliable than the IgM index in detecting a local inflammatory immune response (Sharief *et al.*, 1990).

Using the presence or absence of CSF IgM OCB as the grouping variables, the discriminant power of various clinical and laboratory parameters in MS has been evaluated (Table 4). Although some dichotomous variables such as sex and IgG OCB were included among the predictable variables of the discriminant analysis, the multivariate normality assumption required for applying discriminant analysis has not been violated. The equality of the group covariance matrices has been tested by Box's M test, results of which are shown in the footnote to Table 4; the significance of 0.26 indicates that the covariance matrices are equal.

As shown in Table 4, 6 different parameters significantly discriminate between MS patients who produce IgM intrathecally and those who do not. Three variables representing disease activity, i.e., disease duration, total number of relapses, and the time interval between the date of the lumbar puncture and the date of the previous relapse, appear to discriminate between the two groups of MS patients. Pleocytosis, CSF kappa and lambda FLC bands are the laboratory variables that potentially discriminate between those patients who show IgM in CSF and those who do not.

TABLE 4 UNIVARIATE EQUALITY AND DISCRIMINANT FUNCTION ANALYSIS* OF VARIOUS VARIABLES TO DISCRIMINATE BETWEEN MS PATIENTS WITH AND WITHOUT CSF OLIGOCLONAL IgM BANDS

Variable	*Wilk's lambda***	*Canonical discriminant function coefficients*		*P*
		Unstandardized	*Standardized*	
Clinical group	0.99909	0.06526	0.07426	0.699
Sex of patient	0.99998	0.04323	0.02093	0.953
Age of patient	0.99715	0.01440	0.17622	0.495
Disease duration	0.87261	0.19032	0.67138	0.002
Time from last relapse	0.82581	0.23815	0.50286	0.001
Number of relapses	0.96021	0.04271	0.31052	0.014
Total number of plaque lesions	0.98733	0.39527	0.16322	0.093
Localization of plaque lesions	0.99937	−0.22911	−0.10737	0.218
Size of plaque lesions	0.99882	0.07433	0.08115	0.755
KDSS	0.98512	−0.22418	−0.03462	0.118
Progression index	0.98922	0.43539	0.14739	0.184
Initial symptom	0.99901	−0.83301	−0.31592	0.361
CSF oligoclonal IgG bands	0.99973	0.66276	0.21397	0.834
CSF IgG index	0.99985	−1.02739	−0.62037	0.465
CSF free lambda bands	0.87261	0.19032	0.67138	0.002
CSF free kappa bands	0.90563	0.21356	0.26088	0.003
CSF leucocyte count	0.95176	0.03897	0.36053	0.004
CSF total protein	0.99513	−0.00337	−0.17334	0.373
	(Constant)	1.31784		

* Test of equality of group covariance matrices using Box's M, Box's M = 1.33, approximate F = 1.28, significance = 0.26 ** Wilk's lambda: the ratio of the within-groups sum of squares to the total sum of squares.

The 6 variables mentioned above in fact, correctly predicted group membership of MS patients with regard to the intrathecal production of IgM in almost 81% of the cases (Table 5). Including additional variables to the discriminant analysis decreased the percentage of cases classified correctly, indicating that some of the variables included in Table 4 are poor indicators of intrathecal production of IgM in MS.

It is of interest that total number of plaque lesions or their size or location exhibited no correlation with intrathecal oligoclonal IgM synthesis. Moreover, lesion size as well as total area of lesions in the cerebrum, cerebellum, and brainstem failed to correlate with total quantity of IgM synthesized intrathecally as calculated by the amount of CSF IgM or IgM index value (F = 2.16, $P = 0.093$ and F = 3.24, $P = 0.076$, respectively).

Correlation of intrathecal IgM production with clinical features of MS

Disease duration. A negative correlation was found between the presence of IgM OCB in CSF and a long disease duration. Some 73 of 99 patients (74%) with a disease duration less than 5 yrs showed positive CSF IgM OCB, while only 9 of 51 patients (18%) with disease process longer than 5 yrs showed a similar finding (fig. 1). This inverse correlation is statistically significant ($P < 0.001$). Table 6 compares the difference in means of patients who synthesize IgM intrathecally and those who do not when correlated with disease duration, time period between lumbar puncture and last relapse, and number of relapses. Detection of CSF IgM OCB correlated better with disease duration than the quantitative determination of intrathecally synthesized IgM (i.e., IgM index or total CSF IgM content).

TABLE 5. OLIGOCLONAL IgM-POSITIVE PATIENTS CORRECTLY CLASSIFIED BY STEPWISE VARIABLE SELECTION ALGORITHMS OF DISCRIMINANT FUNCTION

Variables included	*Percentage correctly classified*
Disease duration	65.1%
Disease duration, no. of relapses and time from last relapse	74.6%
CSF free light chain bands	68.2%
CSF free light chain bands, pleocytosis	71.7%
Duration, time from last relapse, CSF free light chain bands and CSF pleocytosis	80.7%
All variables listed in Table 4	59.3%

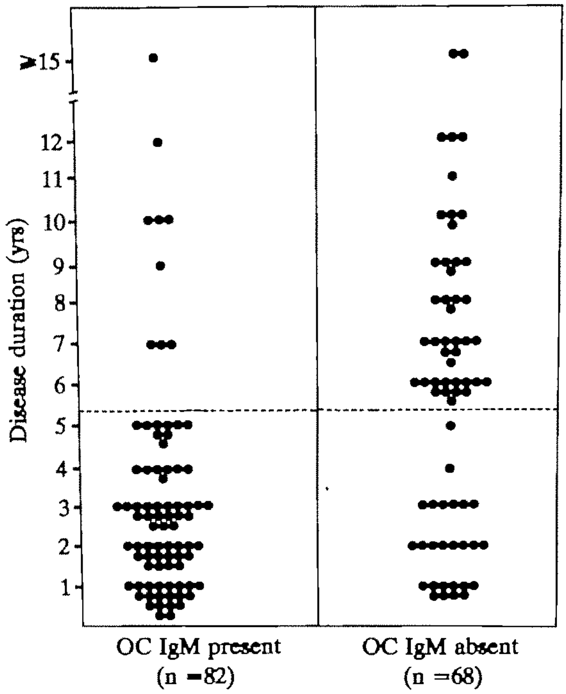

FIG. 1. Plot of disease duration (yrs) versus CSF oligoclonal (OC) IgM.

Disease activity. In patients with relapsing and remitting disease, including those with early probable MS who had had one or more episodes of the disease, a negative correlation was found between CSF IgM OCB and the time interval from lumbar puncture to the last relapse ($P = 0.001$). Of 62 patients who had the lumbar puncture within 2 months of the onset of a relapse, 51 (82%) showed IgM OCB, whereas only 5 of 31 patients (16%) having the lumbar puncture performed more than 2 months after the onset of a relapse showed IgM OCB. A statistically significant correlation was also found between CSF IgM OCB and the total number of relapses during the disease course ($P < 0.01$); the greater the total number, the more likely was the IgM to be oligoclonal. Detection of IgM OCB correlated with the time interval from last relapse and number of relapses more significantly than the IgM index or total CSF IgM amount (Table 6).

TABLE 6. CORRELATION OF INTRATHECAL IgM SYNTHESIS (DETECTED THROUGH DIFFERENT APPROACHES) AND DISEASE DURATION, TIME INTERVAL FROM LUMBAR PUNCTURE TO LAST RELAPSE, AND NUMBER OF RELAPSES

	Difference between means	*df**	*t test statistic*	*95% CI***
Disease duration				
Oligoclonal IgM bands	2.63	148	−5.28	1.64−3.62
IgM Index value	1.10	148	−2.06	0.03−2.17
Total CSF IgM amount	1.18	148	−2.21	0.11−2.25
Time interval from last relapse				
Oligoclonal IgM bands	7.54	133	−8.80	5.84−9.24
IgM Index value	3.03	133	−2.90	2.00−4.16
Total CSF IgM amount	4.03	133	−4.00	2.01−6.05
Number of relapses				
Oligoclonal IgM bands	0.68	134	4.67	0.38−0.98
IgM Index value	0.43	134	2.85	0.13−0.73
Total CSF IgM amount	0.53	134	3.10	0.23−0.83

* Degrees of freedom. ** 95% confidence interval.

Degree of disability and disease progression. The degree of disability in different clinical groups, as determined by the KDSS, has already been described (Table 2). Patients' disability was also evaluated by determining Functional System Grades (Kurtzke, 1965). The association between intrathecal production of IgM and disability status did not reach statistical significance ($P = 0.118$). Similarly, intrathecal production of IgM did not correlate with Functional System Grades ($P = 0.096$) or to the degree of disease progression as manifested by the Progression Index ($P = 0.184$). In fact, intrathecal IgM synthesis failed to correlate with degree of disability even when patients were divided into a low disability group (KDSS 0−3) and high disability group (KDSS 4−7) (Table 7). Oligoclonal IgM bands did not correlate to localization of plaque lesions or initial symptom. Likewise, total CSF IgM or IgM index value failed to correlate with either degree of disability or Progression Index.

TABLE 7 NUMBERS OF PATIENTS WITH HIGH AND LOW DISABILITY SCORE ACCORDING TO THE PRESENCE OF OLIGOCLONAL IgM BANDS IN CSF

Kurtzke disability scale	*Oligoclonal IgM**		*IgM index value**		*Total CSF IgM (mg/l)***	
	Absent	*Present*	*≤ 0.068*	*> 0.068*	*≤ 0.41*	*> 0.41*
0−3	37	47	35	49	30	54
4−7	31	35	31	35	30	36

* $P = 0.13$ ** $P = 0.18$

CSF profile

Leucocytes. The CSF leucocyte count was corrected, according to the peripheral count, if red blood cells were also shown to be present. No polymorphonuclear leucocytes were reported in any patient; all cells were recorded as being mononuclear. Reactive lymphocytes (different in size and staining characteristics due to high cytoplasmic Ig content) and/or plasma cells were mainly detected in CSF specimens with pleocytosis, and were seen in 21 (46%) of 46 IgM OCB-positive and in 22 (39%) of 57 IgM OCB-negative specimens. In none of the groups was the observed difference large enough to reach statistical significance. Similarly, no significant correlation was found between reactive lymphocytes/plasma cells and IgM index or total CSF IgM amount.

The CSF leucocyte count was 5 or more cells/mm^3 in 44 (54%) of the 82 patients with positive CSF IgM OCB (fig. 2). In contrast, only 8 (12%) of the 68 patients who lacked an oligoclonal IgM response in the CSF showed a leucocyte count of 5 or more cells/mm^3. The association between CSF oligoclonal IgM and pleocytosis was statistically significant (corrected $\chi^2 = 22.32$, $P < 0.005$). Similarly, the CSF leucocyte count significantly correlated with IgM index (corrected $\chi^2 = 17.53$, $P < 0.01$); of the 84 patients with high IgM index value, 40 (47%) had 5 or more cells/mm^3 while such a finding was found in 9 patients (14%) of the 66 who demonstrated a normal IgM index value.

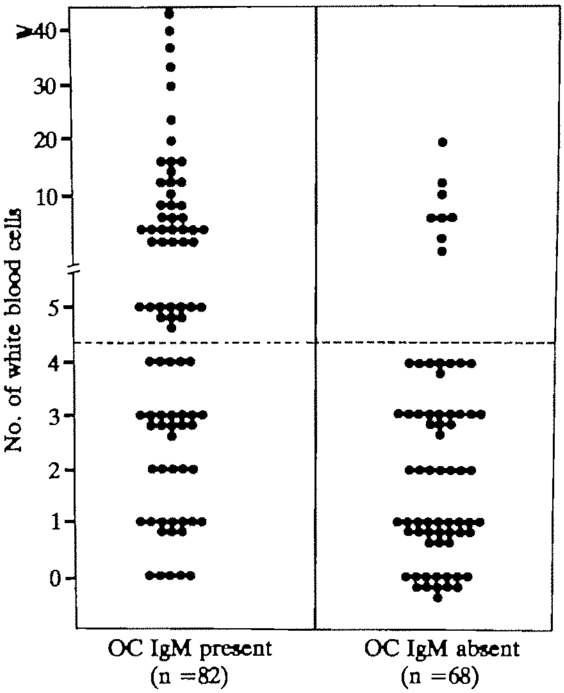

Fig. 2. Plot of number of leucocytes versus oligoclonal (OC) IgM in CSF

Total protein concentrations. CSF total protein ranged from 0.12 g/l to 0.92 g/l (mean ± SD = 0.49 ± 0.28 g/l). No significant association was found between IgM OCB and CSF total protein content (corrected $\chi^2 = 0.68$, $P = 0.83$).

CSF oligoclonal IgG. Of the 150 patients included in this study, 147 (98%) had IgG OCB in their CSF, 81 (55%) of whom were also positive for oligoclonal IgM. One of the 3 patients negative for IgG OCB, a patient with active disease of 9 months duration, demonstrated CSF IgM OCB. The association between oligoclonal CSF IgG and oligoclonal IgM was statistically significant ($P < 0.05$), but there was no correlation between oligoclonal IgM and IgG index ($\chi^2 = 0.89$, $P = 0.23$).

CSF FLC bands. Of the 150 patients examined, 35 (23%) had CSF bands comprising both kappa and lambda FLC. Another 50 patients (33%) had only lambda FLC bands and 4 other patients (3%) demonstrated CSF bands exclusively of kappa type. A significant correlation was found between IgM OCB and lambda FLC bands ($P < 0.001$). Two or more CSF lambda FLC bands were detected in 72% of patients positive for IgM OCB, while 38% IgM OCB-negative patients had lambda FLC bands in CSF.

There was a similar significant correlation between the presence of oligoclonal IgM in CSF and kappa FLC bands ($P < 0.01$); 41% of patients who were positive for CSF IgM OCB had kappa FLC bands in their CSF. In contrast, only 7% of patients negative for IgM OCB had CSF kappa FLC bands. The relationship of IgM OCB to both disease duration and free light chains is shown in Table 8.

TABLE 8 SUMMARY TABLE FOR ANOVA ANALYSIS OF THE EFFECT OF CSF FREE LIGHT CHAIN BANDS AND DISEASE DURATION ON THE PRESENCE OF OLIGOCLONAL IgM IN CSF

Source of variation	*F**	*P*
Free lambda light chain bands	10 096	<0.001
Free kappa light chain bands	5 361	0.025
Log disease duration	32 889	<0 0001
2-way interaction between		
free lambda bands and log disease duration	3 873	0.036
free kappa bands and log disease duration	2.361	0.098
free lambda and free kappa bands	1 325	0 169

$$*F = \frac{\text{between-groups variance estimate}}{\text{within-groups variance estimate}}$$

DISCUSSION

The purpose of this article is to describe the relationship of intrathecal synthesis of IgM, demonstrated by CSF OCB, to various clinical and laboratory parameters in MS. Hitherto, studies on CSF oligoclonal IgM in MS have been mainly concerned with the frequency of such a finding (Keir *et al.*, 1982; Walsh *et al.*, 1985; Gallo *et al.*, 1987). Having established a sensitive and specific method for detecting IgM OCB in native CSF, we embarked on studying the relationship between oligoclonal IgM and other clinical and CSF characteristics of MS.

Detection of intrathecal IgM synthesis

The detection of IgM OCB in the CSF was used as the standard procedure for asssessing intrathecal production of IgM in MS patients. Oligoclonal bands were found to be more specific for active disease than the IgM index or the total CSF IgM amount. This 'qualitative' approach distinguishes the restricted charge characteristics of intrathecally produced IgM, thereby avoiding some of the disadvantages of the quantitative techniques.

Quantitative determinations of intrathecal IgM synthesis utilize albumin as a quantitative marker of blood-CSF permeability and take into account proportional passage of albumin and IgM through blood-CSF barriers. Accordingly, local IgM production in MS was detected through a raised CSF IgM/albumin ratio (Williams *et al.*, 1978) and later through raised IgM index values (Frydén *et al.*, 1978; Sindic *et al.*, 1982; Forsberg *et al.*, 1984). But although the quantitative approach is usually applicable to IgG, it should be interpreted with caution for IgM, partly because of the high serum/CSF ratio, which is around 4000 (compared with only 400 for IgG) and partly because the passage of IgM through the blood-CSF barriers is reported to be disproportionate to that of albumin (Felgenhauer, 1982). In addition, only a slight correlation was found between CSF IgM and serum IgM (Sindic *et al.*, 1982; Forsberg *et al.*, 1984) implying that CSF IgM is regulated in a less predictable manner than that for small proteins. Focal disruption of blood-CSF barrier may therefore contaminate intrathecally synthesized IgM with serum IgM and artificially increase the index value. In fact, the IgM index value was reported to increase dramatically with increasing barrier permeability unlike the IgG index, which particularly remains invariant (Felgenhauer, 1982).

Another problem encountered in the calculation of the IgM index is the occurrence of monomeric IgM which was reported to constitute a large proportion of IgM detected in certain CNS infections, specifically lymphocytic meningoradiculitis (Henriksson *et al.*, 1985). Monomeric IgM influences the results of IgM determination, and hence the IgM index value, while probably having no antibody activity.

The current method of GA-enhanced immunofixation, which utilizes agarose gel electrophoresis, avoids most of the disadvantages of isoelectric focusing (IEF) in detecting IgM OCB. The main limitation is the very low concentration of CSF IgM. On IEF, IgM can be spread out over a disance of 8–9 cm (Keir *et al.*, 1982), while in agarose electrophoresis it is only 3 cm. This difference amounts to a 3-fold concentration of bands on agarose electrophoresis. The limited resolving power of IEF to detect IgM bands may result from the relative insolubility of polymeric IgM under the conditions of low ionic strength required for IEF. Moreover, human IgM, and particularly CSF IgM, is acidic in its range of pI and suffers the low IEF resolution for acidic proteins. Additional problems in IEF are the frequent incidence of artefacts in acidic ampholytes, and fractionation of a monoclonal immunoglobulin into multiple bands (Awdeh *et al.*, 1970). In such a context, agarose electrophoresis seems to be well suited for the accurate detection of IgM OCB.

CSF oligoclonal IgM in MS

The role of immunological processes in the pathogenesis of MS is not understood, but a role for antigenic drive in the aetiology has been considered by many investigators. The presence of intrathecal synthesis of IgM in some patients with MS may indicate a specific response against a recent antigenic stimulation within the CNS by an infectious agent, mainly viral, or an autoantigen. The restriction of CSF IgM in MS is more in favour of a specific immune response which can be sometimes tested with specific antigens.

Another plausible cause of the presence of oligoclonal IgM in CSF might be reactivation of a latent virus within the CNS with a specific IgM antibody response, or it might signify a persistent antigenic challenge within the CNS. Obviously, the detection of intrathecal IgM synthesis in 55% of MS patients underlines the need for further studies of the antigenic specificity of IgM which may reveal the causative antigens responsible for the IgM response.

Oligoclonal IgM and the clinical course of MS

The finding of a negative correlation between CSF oligoclonal IgM and the time period from the last relapse is an argument for new or repetitive antigenic stimulation during exacerbations of the disease state. This argument is further substantiated by the finding that IgM OCB are significantly correlated with the total number of relapses during the course of the disease process. Strong support for this argument would be obtained by determining, through longitudinal studies, whether relapses in the same MS patients are associated with increased occurrence of CSF oligoclonal IgM. Nonetheless, the association with acute relapses makes the hypothesis that MS is a disorder of immune regulation and that immunoglobulin production is 'nonsense antibody' (Mattson *et al.*, 1980) rather unlikely.

The present study provides evidence that intrathecal IgM synthesis is a reasonably good parameter of disease activity in MS. Namely, the significant correlation of oligoclonal IgM with the number of relapses indicates a specific immune response that is related to the development of new lesions and not due to indiscriminate entry of B-lymphocytes into MS brain at any random stage of the disease process. It is tempting to speculate that IgM OCB may reflect the duration and activity of an individual exacerbation of MS since our findings further extend those of Dowling and Cook (1976) where serum IgM levels were found to show close correlation to disease activity (defined as a recent relapse).

The negative correlation of CSF IgM OCB with disease duration is also interesting. The negative correlation with CSF oligoclonal IgM may imply that intrathecal IgM synthesis may vanish in 'burnt-out' cases.

There was no significant correlation between IgM OCB and degree of disability. Oligoclonal IgM failed to correlate with disease disability even when Poser's 'Progression Index' (overall KDSS/duration) was evaluated. If relationships between IgM and extension of the demyelinating process are expected, disability is more likely to be determined by anatomical location than by size. In this context, intrathecal IgM synthesis in MS is clearly shown to have different implications than that of IgG. Unlike IgM, the relative CSF IgG level was found to correlate significantly with cerebral plaque area (Baumhefner *et al.*, 1990), and did not correlate with disease duration (Walker *et al.*, 1985; Müller *et al.*, 1989). Since plaques, particularly chronic ones, are a major site of IgG synthesis (Tavolato, 1975), the association between CSF IgG and degree of disability or total cerebral plaque area indicates that intrathecal IgG production is a manifestation of the accumulation of plaques in the CNS as the disease takes its course. Absence of such a correlation with intrathecal IgM synthesis, on the other hand, implies that CSF IgM is not directly related to chronic plaques and may have resulted from a spillover into CSF from new plaques (Weber *et al.*, 1988).

Oligoclonal IgM and MRI

MRI is the best test for the detection of lesion dissemination in space. However, exact correspondence between actual and MRI lesions is not possible because of volume averaging that occurs in the production of MR images (Paty, 1988). Moreover, tissue characterization is not possible since inflammation and oedema contribute to the MRI appearance of early lesions, whereas demyelination and gliosis contribute to increased signal in chronic lesions (Koopmans *et al.*, 1989). The lack of correlation between intrathecally produced IgM and MRI-detected plaque burden may therefore be due to signal detection from gliosis, remyelination, or regenerating lesions that are not immunologically active. Increased plaque burden may otherwise be due to oedema without concomitant immunoglobulin production.

Oligoclonal IgM and other CSF parameters

There was a strong correlation between oligoclonal IgM and leucocyte count probably because most of CSF IgM is produced by lymphocytes within the intrathecal compartment. Henriksson *et al.* (1981, 1985) demonstrated that CSF lymphocytes function in the protein A plaque assay with IgM production, and IgM-containing cells (mainly plasma cells) were reported to be present in CNS in the acute phase of poliomyelitis but not in the convalescent stage (Esiri, 1980*a*). These IgM-containing cells most probably represent an acute immune response to the invading polio virus. Furthermore, IgM-containing cells were found in more than 80% of CSF from MS patients taken during the acute exacerbation of the disease, and were suggested to be an early marker of the inflammatory process (Weber *et al.*, 1988).

Free light chains have previously been demonstrated in MS CSF (Link, 1967). The present study confirms previous observations that abnormal FLC migrating as bands are found in CSF from MS patients and are mostly of lambda type (Link and Laurenzi, 1979; Laurenzi *et al.*, 1980; Vakaet and Thompson, 1985). We could not find FLC bands in serum from our patients probably due to the high renal clearance of low-molecular weight proteins. The presence of FLC bands may reflect a by-product during antibody production under intense immunological stimulation. Esiri (1980*b*) found a preponderance of either kappa or lambda FLC in some plaques, particularly recent ones, probably indicating specific antigenic challenge; a nonspecific mitogenic effect is more likely to lead to an even balance of light chain types. The pathophysiology of FLC bands in MS CSF has already been discussed (Sharief and Thompson, 1989).

While oligoclonal IgG is of diagnostic value in MS, oligoclonal IgM is likely to be more valuable for investigating the pathogenic mechanism than for routine diagnostic purposes. It is, nonetheless, a reasonably good parameter in evaluating the relapse activity of the disease process. It indicates the activity of intensely stimulated B-lymphocytes which may signify persistent stimulation by an antigen inside the CNS. It could also be used to help monitor longitudinal lumbar punctures of patients with various treatments in line with the hypothesis that following a reduction in antigenic stimulation, the IgM should be the first to return to normal, as is classically seen in the treatment of neurosyphilis.

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(Received May 24, 1989. Revised December 28, 1989. Accepted February 1, 1990)

Brain (1991), 114, 197–214

AXIAL MYOCLONUS OF PROPRIOSPINAL ORIGIN

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SUMMARY

Three patients are described with nonrhythmic repetitive axial myoclonic jerks causing symmetric flexion of the neck, trunk, hips and knees. No electrophysiological evidence of a cortical or brainstem reticular origin for the myoclonus was found. In the first patient the axial jerks only occurred spontaneously. The latencies of recruitment of spinal segments during a jerk indicated that the discharge arose in the mid thoracic cord and then slowly spread at about 5 ms^{-1} up and down the cord to involve rostral and caudal segments. No structural lesion was identified in this patient. In the second patient spontaneous and reflex axial jerks developed following the excision of a cervical haemangioblastoma. In the stimulus-induced jerks the relative latencies of muscles innervated by rostral and caudal spinal segments suggested that the myoclonus originated between the upper cervical and midthoracic cord. In the final patient, EMG activity during spontaneous and stimulus-induced jerks commenced in the rectus abdominis, and was followed by later activity in muscles innervated by rostral spinal segments, suggesting that the myoclonus originated in the midthoracic cord. No structural lesion was identified in this patient. Electrophysiological evidence is used to argue a spinal origin for these axial jerks in all 3 cases. There are striking features common both to this form of human myoclonus and to long propriospinal pathways identified in animals. The new concept of propriospinal myoclonus is suggested.

INTRODUCTION

Myoclonus may arise from generators in many sites within the nervous system. Cortical myoclonus is usually focal or multifocal and is due to discharges in the sensorimotor cerebral cortex which are propagated via corticomotoneuron pathways (Shibasaki *et al.*, 1978; Hallett *et al.*, 1979; Obeso *et al.*, 1985). Brainstem reticular myoclonus causes generalized body jerks via slower conducting reticulospinal pathways (Hallett *et al.*, 1977; Shibasaki *et al.*, 1988). Brainstem generators may also activate cortical discharges (Thompson *et al.*, 1989), possibly via the thalamus (Milhorat, 1967), to produce focal or multifocal jerks. In addition to these cerebral mechanisms generating myoclonus, focal or segmental myoclonus may also be caused by local spinal generators (Halliday, 1967), or lesions of the spinal roots (Sotaniemi, 1985), cervical or lumbosacral plexus (Banks *et al.*, 1985), or peripheral nerves (Said and Bathien, 1977).

Spinal myoclonus, as described so far, typically has been confined to muscles innervated by a few adjacent spinal segments (hence the description as spinal segmental myoclonus), has been rhythmic and has often persisted in sleep (Halliday, 1967). Local spinal pathology, be it infectious (Ellis, 1920; Hopkins and Michael, 1974), inflammatory (Campbell and Garland, 1956), neoplastic (Garcin *et al.*, 1968), traumatic (Lhermitte, 1919) or vascular (Davis *et al.*, 1981), is believed to cause loss of local inhibitory spinal

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interneuronal function, thereby liberating the spontaneous repetitive discharge of local segmental anterior horn cell pools to cause focal myoclonic jerks (Howell *et al.*, 1979).

The spinal cord, however, possesses not only local segmental organization, but also powerful and complicated long propriospinal pathways linking activity in many segments. Abnormal activity in these propriospinal systems could, conceivably, generate a more complicated and more extensive form of spinal myoclonus. Here we present evidence from 3 patients for the existence of such propriospinal myoclonus in man. It is characterized by repetitive, usually flexor and nonrhythmic jerks of the neck, trunk, both hips and knees.

METHODS

Neurophysiological studies included routine electroencephalography (EEG), polymyography, back-averaging of the EEG activity preceding spontaneous jerks and somatosensory evoked potentials (SEPs). Electromyographic (EMG) recordings were made using bipolar silver/silver chloride electrodes placed 2 cm apart longitudinally over the muscle bellies. Those muscles most regularly involved and with well defined bursts of EMG activity in each jerk were selected to trigger the collection of EEG data for back-averaging. The same muscles were used to trigger the collection of EMG data from other muscles to define their order of recruitment during jerks. The latter was calculated from single trials rather than averaged data.

SEPs were recorded following stimulation of the tibial nerve at the ankle with a constant voltage rectangular pulse of 0.3 ms duration at intensities just above motor threshold. SEPs were the average of 128–256 stimuli and their amplitude was measured from the first major cortical positive peak following the stimulus (conventionally known as the P_{40}, here termed P_1) to the second major negative peak (the N_{50}, here termed N_2). Controls (14 healthy subjects, aged 18–40, mean 27 yrs) had a mean P_1 latency of 40.4 ms (range 35.8–44.1 ms) and a mean P_1N_2 amplitude of 3.3 μV (range 0.5–11 μV) with stimulation of the tibial nerve.

The EEG was recorded from silver/silver chloride electrodes fixed to the scalp with collodion and referred to linked ear-lobe electrodes. The signals were band pass filtered at 2.5 kHz with a time constant of 1.0 s, and data were collected by a PDP 12 computer. The EEG recording sites were standard positions of the 10–20 system with additional electrodes used between these sites for the localization of focal cerebral activity, as necessary. In the EEG records shown, positivity is downward.

Magnetic stimulation of the cortex or spinal roots (Ugawa *et al.*, 1989) was performed with a 9 cm diameter stimulator coil using a Novametrix Magstim 200. Spinal segmental lengths were calculated using data derived from postmortem examinations (Donaldson and Davis, 1903).

CASE HISTORIES

Case 1

A 47-yr-old man spontaneously developed involuntary generalized jerks of his body with flexion at the knees, hips, trunk and neck. The frequency and severity of the jerks increased when lying flat, but the jerks were evident on sitting or standing, and to a lesser extent when walking. They did not interfere with gait and did not make him fall. The jerks also occurred during sleep. They were not preceded by a build-up of 'inner tension' and could not be suppressed by an effort of will. The strength of the arms and legs was normal, and there was no sphincter or sensory disturbance. In the past the patient had been in good health with the exception of a 6 yr history of low lumbar back pain and right sciatica cured by a L5/S1 discectomy 3 yrs before the development of the truncal jerks.

Over the ensuing 2 yrs the jerks occurred every few minutes despite trials of baclofen 30 mg daily, sodium valproate 800 mg daily and milacemide 2400 mg daily. The combination of clonazepam 1 mg daily and temazepam 60 mg nocte was of modest benefit.

General examination early in his illness was normal. However, when seated or lying there were sudden simultaneous flexion movements of the neck, trunk, hips and knees. These could not be provoked by cutaneous stimuli or tendon taps. The jerks were most violent on lying flat when they would momentarily bring the knees onto the chest. They were less frequent and less marked on sitting and standing, and did not interrupt walking The cranial nerves, muscle tone, tendon reflexes, abdominal reflexes and strength were normal, but initially the plantar responses were extensor. Sensory examination, including over the trunk and buttocks, was normal. These findings remained unchanged over the ensuing 4 yrs, with the exception of the plantar responses which became flexor. Investigations including cerebrospinal fluid examination, EEG and computerized tomography (CT) of the brain were normal.. Myelography showed a very small C5/6 disc protrusion without cord compression.

Case 2

A 40-yr-old man first noticed paraesthesiae in the fingers of the right hand. Over the next 3 yrs the paraesthesiae spread to involve the right leg and right side of the face and head. He had a history of ankylosing spondylitis and gout, but no relevant family history.

When first seen, 2 yrs after the onset of symptoms, abnormalities on examination were limited to brisk upper limb tendon reflexes and reduced pin prick and temperature sensation in the right hand and leg. Myelography showed an extensive cervical syrinx, extending from the craniovertebral junction to T4. Vertebral angiography revealed an intradural mass at the level of C2. At subsequent operation a haemangioblastoma was completely removed.

After the operation he developed brief involuntary flexion jerks involving the trunk and, if severe, the neck, legs and right arm. These were more frequent on standing. The jerks were not preceded by any build up of 'inner tension' and he could not control them. They partially improved when taking carbamazepine 800 mg daily. Six months later he developed extension jerks of the right knee, particularly when turning the head to the left. Over the next 18 months he deteriorated still further with flexion jerks occurring every few minutes and with progression of the right sided paraesthesiae.

Examination at this time revealed no evidence of retinal angioblastoma on fundoscopy and normal cranial nerves. The right brachioradialis and both knee tendon jerks were brisk. The right plantar response was extensor and there was hyperpathia over the perineum and right side of the face. Pin prick sensation was diminished below both knees. Magnetic resonance imaging (MRI) showed backward displacement and expansion of the posterior aspect of the upper cervical cord but no syrinx (*see* fig. 5). Vertebral angiography showed no evidence of tumour recurrence and subsequent surgical exploration found and excized adhesions between the posterior cervical cord and dura mater. After the operation there was marked reduction in the frequency of both the generalized flexion jerks and the extension jerks of the right knee. The paraesthesiae were unchaged.

The examination findings remained largely unchanged 1 yr after the surgical division of the upper cervical adhesions. The spontaneous truncal flexion jerks occurred 5–15 times a day, but now, for the first time, taps to both right and left biceps and patellar tendons elicited brisk, but small, axial flexion jerks.

Case 3

A 32-yr-old man spontaneously developed flexion jerks of his trunk which occurred only when lying down and persisted during sleep. At times they were accompanied by noises due to the forced expiration produced by vigorous abdominal contraction. There was no limb weakness, sensory, sexual or sphincter disturbance. He was otherwise healthy with no personal or family history of significance.

General and neurological examination was entirely normal except that when lying down the patient developed irregular shock-like contractions of axial muscles producing flexion of the trunk, neck and hips. The jerks could be elicited by light touch over the anterior abdominal wall and by tapping the left rectus abdominis with a tendon hammer. His condition remained unchanged over the ensuing 5 yrs. Medical treatment was declined.

RESULTS

Case 1

Truncal jerks occurred irregularly every 20 to 60 s during polymyography in the supine position. They involved the sternocleidomastoid, rectus abdominis, quadriceps, hamstrings and to a lesser and more variable extent the gastrocnemius, tibialis anterior, biceps and deltoid muscles. EMG bursts were small in erector spinae and pectoralis major. The duration of EMG bursts was variable ranging from 100 to 1400 ms in rectus abdominis. Homologous muscles were activated synchronously bilaterally and cocontraction was evident in agonist and antagonist muscle pairs (figs 1, 2).

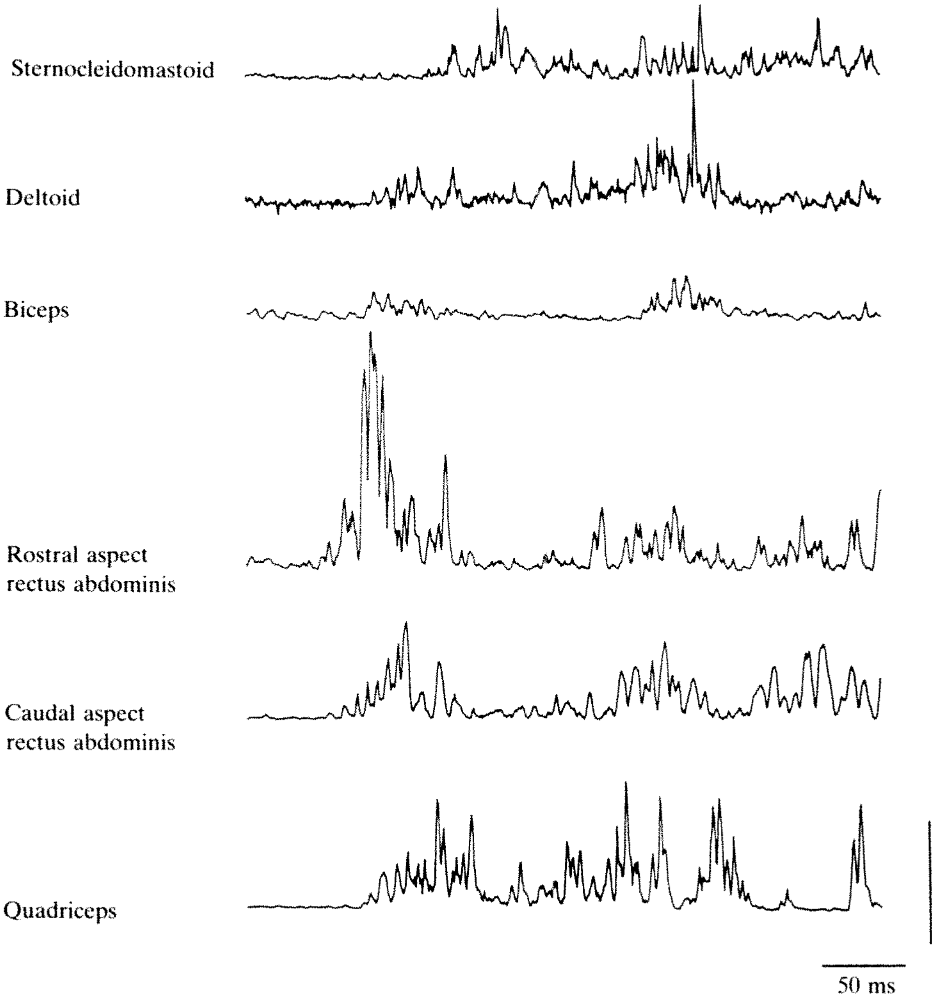

FIG. 1. EMG record of a single spontaneous jerk in *Case 1*. Muscles are sternocleidomastoid, deltoid, biceps, rostral aspect of rectus abdominis, caudal aspect of rectus abdominis and quadriceps. Contraction of sternocleidomastoid, rectus abdominis and quadriceps occurred in all jerks, but activity in deltoid and biceps (evident here) was variable. Vertical calibration line = 200 μV and 100 μV for bottom and top 3 channels, respectively.

There was considerable variability in the relative latencies of individual muscles in the jerks (Table). Despite this, the jerks tended to show a characteristic electromyographic pattern (fig. 1). Within each jerk, the first EMG activity to be recorded was in the superior portion of rectus abdominis. EMG activity in the inferior portion of rectus abdominis

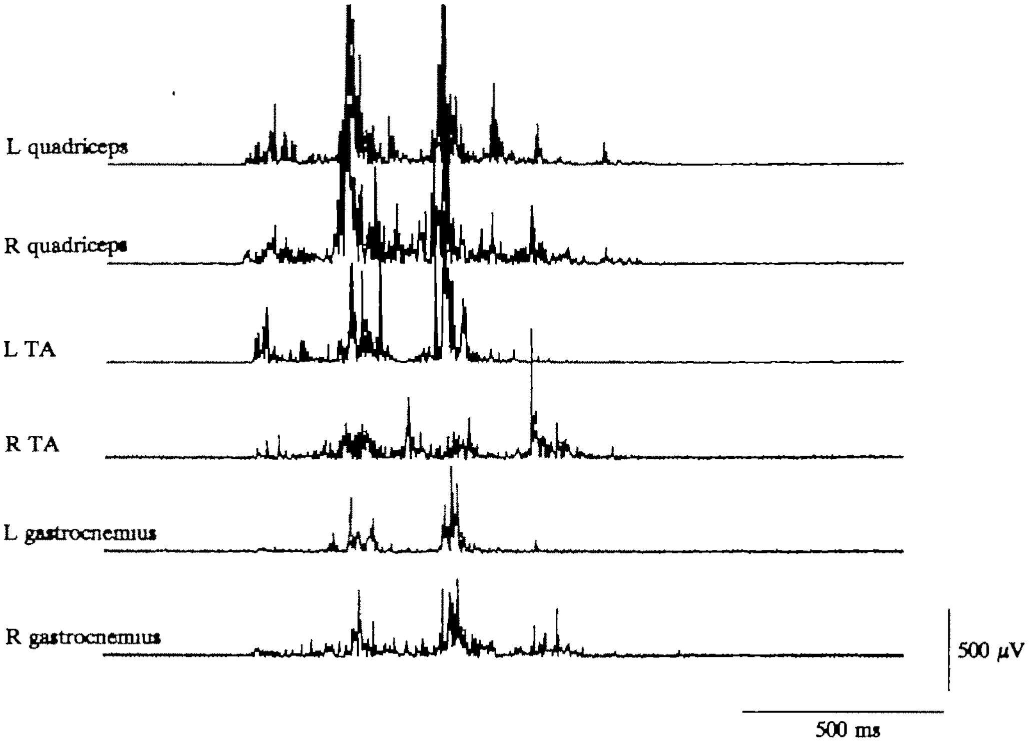

FIG. 2 EMG record of a single spontaneous jerk in *Case 1* showing cocontraction of agonist and antagonist muscles on both sides. Muscles are left (L) and right (R) quadriceps, tibialis anterior (TA) and gastrocnemius.

TABLE RELATIVE LATENCIES OF MUSCLES INVOLVED IN JERKS IN CASE 1*

Muscle	*n*	*Mean (ms)*	*SD*	*Efferent (ms)*	*Spinal delay (ms)*	*Spinal root*	*Distance (cm)*
L sternomastoid	51	35.2	17.1	5.2	39.9	C1, 2	21.2
L deltoid	19	37.2	4.8	6.5	40.6	C5, 6	16.9
L biceps	28	30.6	18.5	6.8	33.7	C5, 6	15.6
L superior rect	49	2.9	9.1	10.3	2.5	T7	0.0
L middle rect.	34	8.9	18.7	9.9	8.9	T10	7.2
L inferior rect.	47	8.5	7.5	9.4	9.0	T12	11.4
R quadriceps	83	20.6	10.0	9.0	21.5	L3, 4	16.7
L quadriceps	83	21.9	10.0	9.0	22.8	L3, 4	16.7
L tibialis ant.	33	34.6	8.0	12.0	32.5	L4, 5	17.2
L hamstrings	20	50.5	16.1	9.9	50.5	S1	18.6
L gastrocnemius	34	46.9	9.3	12.0	44.8	S1, 2	19.4

* n = number of jerks. Mean = mean latency relative to the superior aspect of the right (R) rectus abdominus (Rect.), the SD is given to illustrate the jitter in the relative latencies. Efferent = peripheral efferent delay calculated from magnetic stimulation of spinal roots. Spinal delay = estimated extra time for the recruitment of spinal segments relative to T7 (the spinal segment supplying the superior portion of right rectus abdominus, the muscle with the earliest recorded activity in a jerk). Spinal delay for each muscle was calculated as: (latency relative to superior aspect of right rectus abdominis) minus (efferent conduction time to each muscle, less 9.9 ms which was the efferent conduction time to superior aspect of right rectus abdominis). Spinal root = principal segmental innervation of each muscle. Distance = distance of the principal spinal segments supplying each muscle from T7, calculated from postmortem data (Donaldson and Davis, 1903)

followed about 8.5 ms later ($P < 0.001$). EMG activity was then recorded in muscles both cranial and caudal to rectus abdominis. The latencies of these muscles (relative to the superior portion of rectus abdominis) increased with their distance from the superior portion of rectus abdominis both up and down the body.

It was possible to estimate the conduction velocity of the spinal efferent pathways involved in this form of myoclonus from the relative latencies of the individual muscles (Table). Peripheral efferent times were calculated for each muscle using magnetic stimulation of nerve roots, the coil being placed over the appropriate spinal segment. These peripheral efferent times, less 9.9 ms (the peripheral efferent time for the superior portion of right rectus abdominis), were then subtracted from the delay in onset of EMG activity in each muscle relative to the superior portion of rectus abdominis, to give the order of recruitment (spinal delay) of the spinal segments innervating individual muscles involved in the jerks. When the spinal delay for each muscle was considered as a function of the distance of its innervating segment along the cord from T7 (the segment innervating the superior portion of rectus abdominis) a highly significant positive correlation was found ($r = 0.882$, $P = 0.0001$). The slope of the regression line was consistent with a conduction velocity of about 5 ms^{-1} in spinal efferent pathways leading up and down the cord from T7. This calculation of the conduction velocity was only approximate as it was based on average segmental lengths in postmortem specimens (Donaldson and Davis, 1903) and included delays at both root and synaptic levels. The latter will include the time necessary for temporal facilitation, which is likely to increase with increasing distance from the spinal myoclonic generator, leading to a further underestimate of the true conduction velocity in the spinal efferent pathways involved in the myoclonus. (A similar calculation was made using an estimate of the spinal delay derived from the minimum relative latencies of each muscle. There was a significant correlation between this minimum spinal delay and the distance of segmental innervations from the midthoracic spine ($r = 0.710$, $P = 0.01$). The slope of the regression line was consistent with a conduction velocity of about 6.5 $m{\cdot}s^{-1}$ in the spinal efferent pathways involved in the myoclonus.)

The effect of the jerks on tonic voluntary EMG was investigated, the patient being instructed to keep the right leg relaxed and the left leg extended in the air (producing a moderately steady EMG activity 10–20% of maximum). In the left tibialis anterior, tonic voluntary EMG activity was reduced for 150 (SEM ± 24) ms starting 35 (± 9.6) ms before the EMG burst in the right tibialis anterior, and in the left quadriceps tonic EMG activity was reduced for 72 (± 7) ms starting at the same time as the EMG burst in the right quadriceps (fig. 3).

There was no evidence that the jerks were stimulus sensitive. Cortical SEPs from stimulation of the right and left posterior tibial nerves at the ankle were of normal latency (P_1 at 41 and 42 ms, respectively), and amplitude (P_1N_2 2.8 and 1.3 μV, respectively). Cutaneomuscular reflexes to a train of stimuli delivered to the right hallux habituated normally. Back-averaging the EEG activity in the 125 ms before each jerk did not reveal any short latency time-locked cortical activity. Magnetic stimulation of the cortex produced EMG responses of normal latency in biceps (10 ms), rectus abdominis (20 ms) and quadriceps (22 ms).

Finally, the possibility that the jerks were voluntary was investigated. Back-averaging

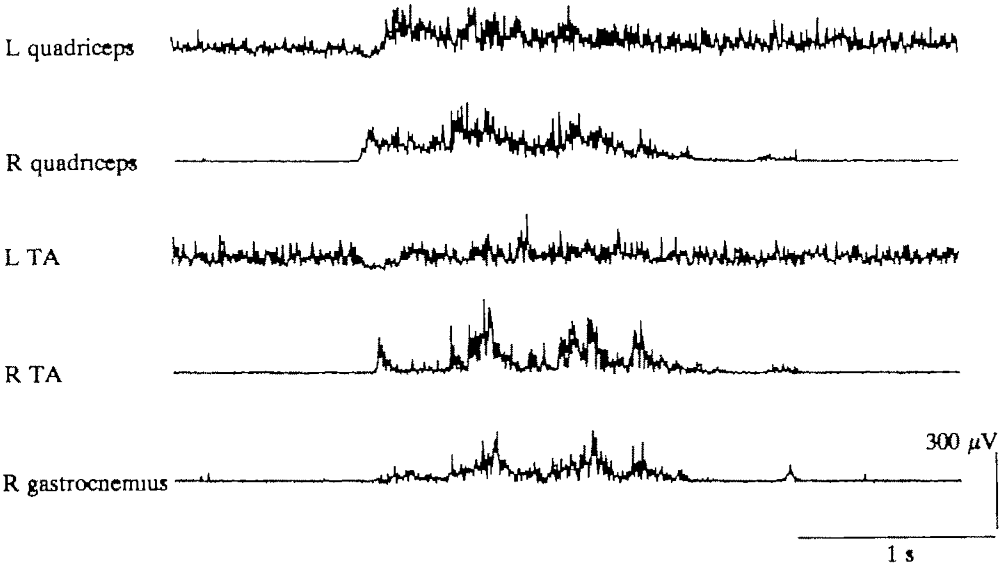

FIG. 3 Average of 10 trials showing the effect of spontaneous jerks on tonic voluntary EMG activity in the left leg in *Case 1* The patient voluntarily lifted the left leg off the bed, while keeping the right leg relaxed. The jerk of the elevated leg is prefaced by a brief inhibition of EMG activity in the left quadriceps and tibialis anterior, coincident with the onset of the EMG burst in the right quadriceps, tibialis anterior and gastrocnemius Muscles are left (L) and right (R) quadriceps, tibialis anterior (TA), and gastrocnemius.

the EEG activity in the 2 s before each spontaneous jerk did not reveal any premovement potential, although a normal premovement potential was recorded before voluntary trunk flexion (fig. 4). When normal subjects were asked to mimic the truncal flexion jerks they exhibited a very different picture of muscle activation to those in Case 1. The muscle activity in agonists and antagonists tended to alternate as seen in normal ballistic limb movements. In addition, unlike Case 1, the superior and inferior aspects of the abdominal recti contracted synchronously. Finally in mimicked jerks, EMG activity was not usually recorded first in the rectus abdominis, and no significant correlation was found between the spinal delay of various muscles and the distance of the respective innervating spinal segments from T7.

Conclusion. The jerks were involuntary. No evidence for a supraspinal origin for the myoclonus was found. The recruitment of muscles within the jerks was determined by the distance of their segmental supply from T7, suggesting a spinal myoclonic generator at about this level.

Case 2

The first opportunity to examine this patient electrophysiologically occurred the day before his surgical reexploration. He was treated preoperatively with dexamethasone 4 mg daily which had greatly reduced jerk frequency so that polymyography (fig. 6A) only recorded 4 jerks. These occurred irregularly over a period of 1 h and involved rectus abdominis, erector spinae and usually quadriceps and tibialis anterior synchronously

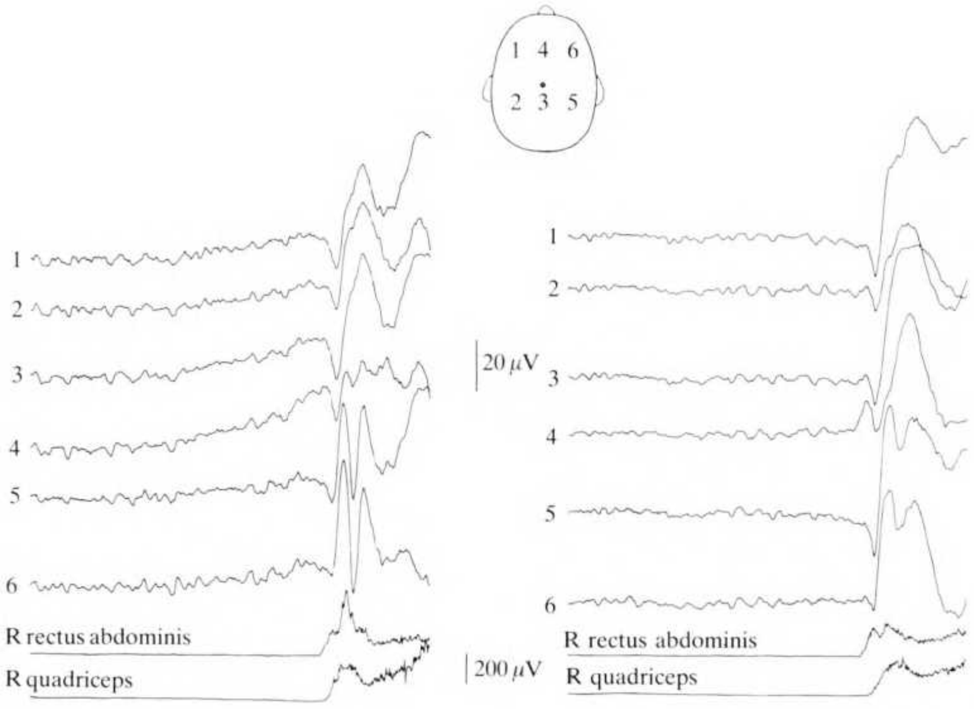

FIG. 4. Back-averaging of EEG activity over 2 s preceding voluntary truncal and hip flexion movements mimicking the spontaneous myoclonic jerks (*left*, average of 88 recordings) and spontaneous jerks (*right*, average of 100 recordings) in *Case 1*. A Bereitschaftspotential is evident preceding the voluntary movements, but not the spontaneous jerks. The postmovement potentials are probably caused by movement artefact. The sweep time is 2.5 s.

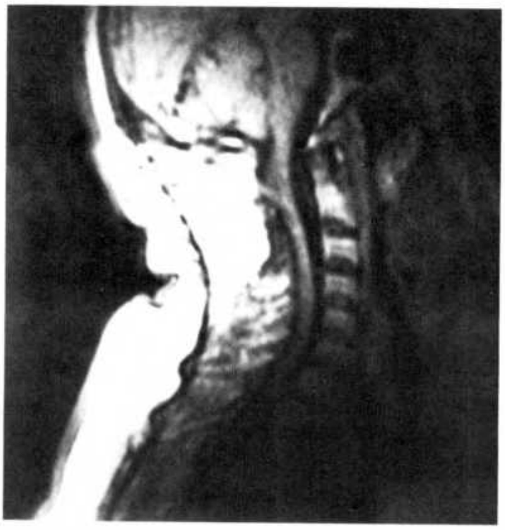

FIG. 5. MRI of the cervical cord in *Case 2* (sagittal spin echo image with gadolinium enhancement), showing backward displacement and expansion of the posterior aspect of the upper cervical cord.

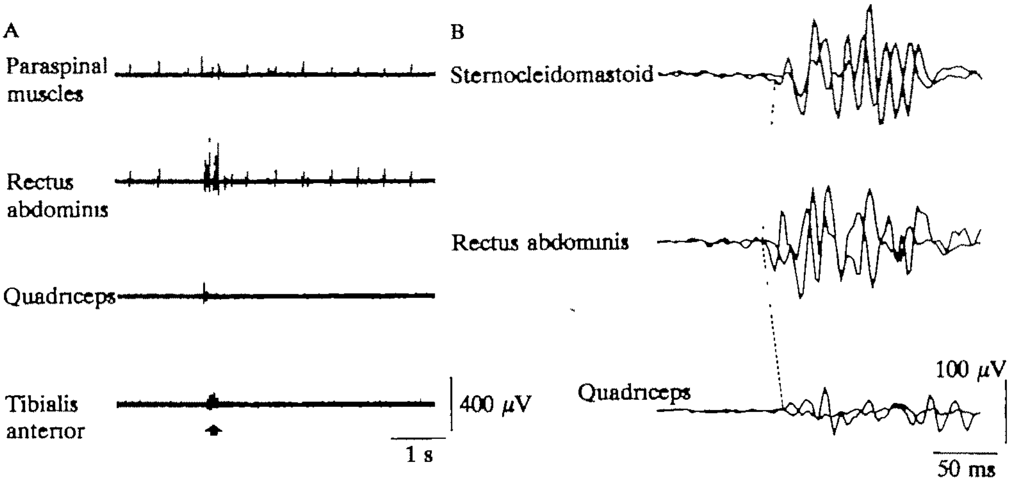

FIG. 6. A, single spontaneous jerk in *Case* 2. The first 2 channels show ECG artefact and there is baseline 50 Hz artefact in all 4 channels. The jerk is marked by an arrow B, 2 superimposed unrectified records of the reflex jerks following taps to the left patellar tendon in *case* 2. EMG activity is recorded first in rectus abdominis and later in sternocleidomastoid and quadriceps femoris. The traces have been started 50 ms after the delivery of the stimulus. In both A and B the illustrated muscles are right sided.

on both sides. Bursts of muscle activity lasted 40–200 ms, with cocontraction of the abdominal recti and erector spinae. There was no stimulus sensitivity at this initial examination. Cortical SEPs from stimulation of the tibial nerves at both the right and left ankles were delayed (P_1 at 46 and 49 ms on right and left tibial nerve stimulation, respectively), but of normal amplitude (P_1N_2 of 0.6 and 0.5 μV, respectively). Back-averaging was precluded by the low frequency of the jerks. Magnetic stimulation of the cortex elicited responses of normal latency in the right tibialis anterior (31 ms) and right quadriceps (28 ms). No responses could be obtained on the left.

The patient was reexamined electrophysiologically 1 yr later. At this time taps to both biceps and quadriceps tendons elicited brief axial flexion jerks. During these, EMG activity was recorded in sternocleidomastoid, rectus abdominis, quadriceps and often in pectoralis major. There was considerable jitter in the relative latencies of muscles involved in the jerks. Despite this jitter, EMG activity was recorded first in rectus abdominis (137.9 ± 5.8 ms, $n = 18$), and significantly later in sternocleidomastoid (148.8 ± 3.7 ms, $n = 16$, $P = 0.014$) and quadriceps (149.8 ± 4.3 ms, $n = 13$, $P = 0.022$) following taps to the left patellar tendon (fig. 6B). The duration of EMG activity during these reflex jerks was brief (50–200 ms).

Conclusion. The patient developed spontaneous axial myoclonic jerks, very similar to those seen in Case 1, following the excision of a cervical haemangioblastoma. The myoclonus worsened with subsequent distortion of the cervical cord by dural adhesions seen on MRI and confirmed during surgical exploration. The spontaneous jerks greatly improved following division of the adhesions. In the reflex jerks that developed after the second operation, the EMG activity was recorded first in the abdominal recti, and later in more rostral and caudal muscles. We conclude, therefore, that the jerks were spinal in origin.

Case 3

During polymyography, jerks occurred irregularly every 20–50 s. There was bilateral and relatively synchronous activation of thoracic and lumbar paraspinal muscles, rectus abdominis, pectoralis major, deltoid and, less often, quadriceps and hamstrings. The duration of EMG bursts was variable, ranging from 100 to 400 ms in rectus abdominis.

In both spontaneous and reflex myoclonus there was considerable jitter in the relative latencies of muscles involved in the jerks. Thus, in spontaneous jerks, EMG activity in the lumbar paraspinal muscles occurred from 12 to 44 ms (mean 26.2 ms) after that in the abdominal recti. Despite this jitter, EMG activity was always recorded first in the abdominal recti (fig. 7). The EMG activity in rostral muscles occurred later (the mean (±SEM) latencies of pectoralis major and deltoid relative to rectus abdominis in 22 jerks were 19.8±2.6 ms and 30.2±1.8 ms, respectively. Too few spontaneous jerks involving the lower limb muscles were recorded to comment on the relative latencies of these muscles).

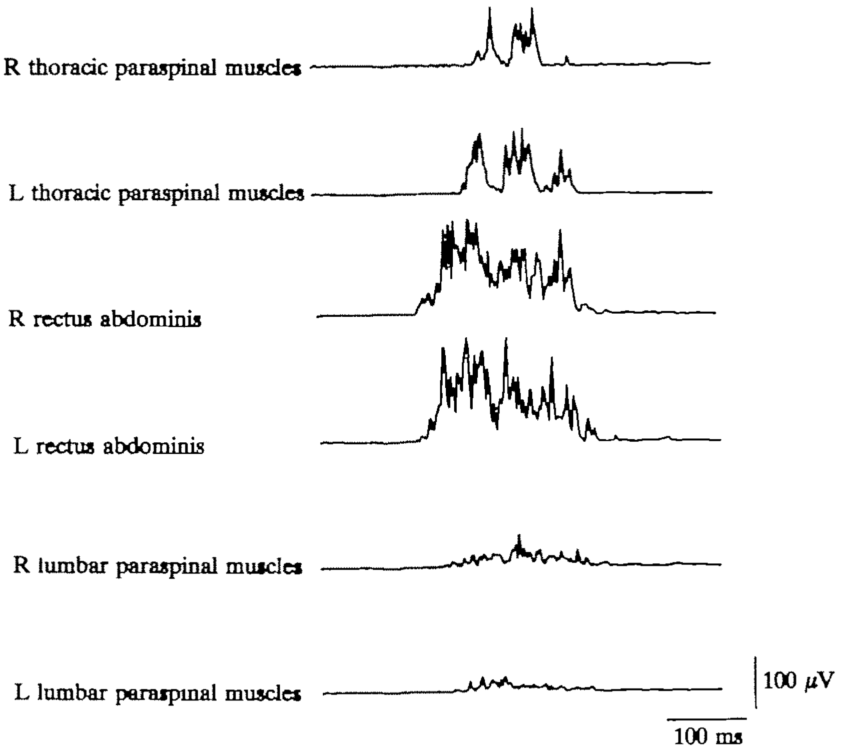

FIG. 7 EMG of a single spontaneous jerk in *Case 3* The first muscles to contract are the abdominal recti

Single taps with a tendon hammer to the anterior abdominal wall elicited several bursts of activity in the abdominal recti. Taps to the left abdomen were unique in eliciting an early burst of EMG activity in the left rectus abdominis at 33.0±1.9 ms, n = 9 (fig. 8). This was separate from the earliest burst of activity seen at about 19 ms in some trials (not evident in fig. 8), which represented the tendon reflex in this muscle. In addition, taps to either the right or left abdomen elicited a series of decreasing bursts of activity in both abdominal recti at 106.0 ± 1.8, 206.7 ± 2.4, 287.8 ± 4.2 and 360.0 ± 6.0 ms. The duration of the first EMG burst in this series was 70.8 ± 2.7 ms (n = 15).

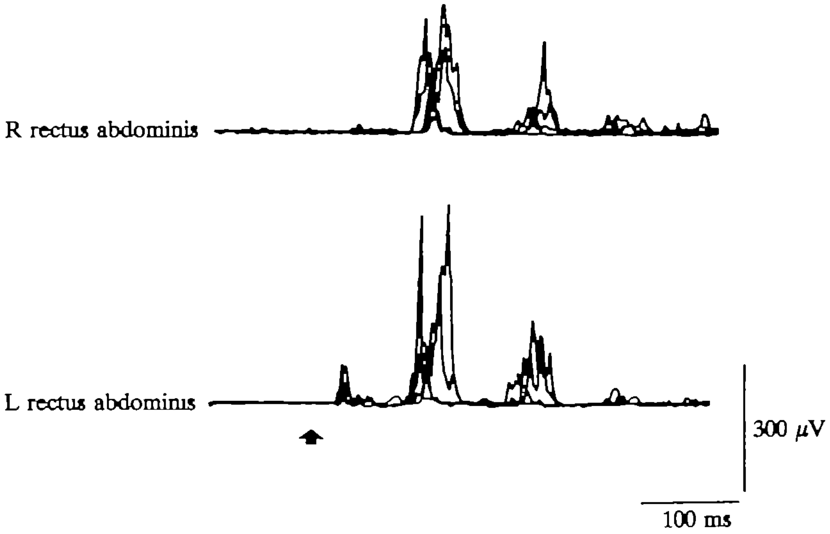

FIG 8 EMG of 5 superimposed trials with a tap (given at 100 ms at arrow) to the left abdomen with a tendon hammer in *Case 3*. The tap sometimes evoked a monosynaptic response in left rectus abdominus (at about 19 ms), but not in this series. The tap then sometimes evoked a response at about 33 ms in left rectus abdominus, evident as the first EMG burst on the *lower trace*. The tap always evoked a later response in both recti at about 106 ms, followed by smaller and less consistent repetitive discharges

Taps with a tendon hammer to either the right or left patellar tendon elicited single bursts of activity in the abdominal recti (at 113.0 ± 5.3 ms, n = 12 for taps to the right patella), and significantly later in the right pectoralis major (at 127.6 ± 5.7 ms, n = 8 for taps to the right patella, $P < 0.001$). The difference between the latency of right pectoralis major and rectus abdominis in spontaneous jerks did not differ significantly from that in jerks elicited by taps to either patellar tendon. The duration of the burst of activity in rectus abdominis to taps to the right patellar tendon was 57.2 ± 3.8 ms (n = 12). Reflex jerks did not involve the lower limbs.

The cortical SEP from stimulation of the right tibial nerve was of normal amplitude (P_1N_2 = 8 μV) and latency (P_1 at 41 ms).

Conclusion. A spinal myoclonic generator seems highly probable in Case 3. In both spontaneous and reflex jerks, EMG activity was recorded first in the abdominal recti, and later in more rostral muscles. Although the most marked reflex responses were, as in Case 2, of long latency, comparable (Roby-Brami and Bussel, 1987) or longer

(Shahani and Young, 1971) spinal reflex latencies have been reported in patients with cord transections. In addition, taps to the left abdomen elicited a response in the left rectus abdominis at 33 ms. Such a short latency response (only 14 ms longer than the probable monosynaptic tendon reflex in the abdominal recti) is incompatible with a cortical reflex loop. Taps to the abdomen may elicit an abdominal skin reflex (Kugelberg and Hagbarth, 1958), but the latter usually has a longer (Duensing, 1940) and much more variable (Kugelberg and Hagbarth, 1958) latency than the reflex elicited in Case 3. In addition, the abdominal skin reflex is readily elicited by electrical stimulation of the skin (Kugelberg and Hagbarth, 1958). The early response in the left rectus abdominis was absent on electrical stimulation of the abdomen.

DISCUSSION

Clinical and electrophysiological features

Three cases with an unusual, distinctive and clinically similar movement disorder are presented. Each exhibited jerks resulting in shock-like flexion at the trunk, hips and often knees and neck. The jerks developed in middle age. In Case 2 jerks developed following excision of a cervical haemangioblastoma and subsequent arachnoiditis, and improved following the surgical division of dural adhesions. In the other 2 cases no definite cause was identified. In these patients no other neurological abnormalities developed except for the bilateral extensor plantar responses present in Case 1 on first presentation alone. The course in these 2 patients was relatively benign, with the myoclonus remaining unchanged in nature for up to 5 yrs. All the patients were active and independent, and only 1 was unable to work (Case 1).

The jerks were very similar in all 3 patients. They consisted of irregular symmetric, relatively synchronous bursts of muscle activity occurring up to twice per second, with a duration varying between 40 ms and 2 s in the abdominal recti, lumbar and thoracic paraspinal muscles, quadriceps, hamstrings and often in the sternocleidomastoids, biceps, deltoids, glutei, tibialis anterior and gastrocnemius. The contraction of flexor muscles, particularly the abdominal recti, dominated. As a result a typical jerk caused flexion of the trunk and often also of the neck, hips and knees. Jerks were most marked on lying down in 2 patients (Cases 1, 3) and in this position the jerks produced a violent movement bringing the flexed knees towards the chest. The jerks often occurred in sleep. In 2 of the 3 patients (Cases 2, 3), the jerks were stimulus sensitive.

Differential diagnosis

The jerks were myoclonic in appearance. The jerks were involuntary and were not preceded by a premovement potential in Case 1. In addition, during the jerks there was cocontraction of agonist and antagonist muscles (e.g., the abdominal recti and paraspinal muscles) in Cases 1, 2 and 3. This was quite unlike the alternating contraction seen in these muscles when normal subjects voluntarily mimicked such movements. Accordingly, we concluded that this myoclonus was real and not psychogenic.

At no time did any of the 3 patients exhibit any sustained abnormal postures that might have suggested that the jerks were part of a dystonic syndrome (Obeso *et al.*, 1983).

Nor did they have any evidence of dystonia elsewhere. Axial torsion dystonia can produce flexor or extensor spasms of the trunk, but these typically appear on action, especially on walking, and decrease or disappear when lying flat or relaxed. The converse was the case in 2 of the 3 patients described here.

On clinical grounds the jerks were also unlikely to represent tics. They did not vary or migrate to involve other parts of the body, they were not preceded by any of the subjective feelings associated with tics, and they were not suppressible (Jankovic, 1987). There were no vocalizations or behavioural disturbances. The absence of any family history and the onset in middle-life would also be unusual. Stimulus sensitivity is not a feature of dystonia or tic disorders.

For these reasons we conclude that these 3 patients had a peculiar type of axial myoclonus. The question then is where did it come from? There was nothing to suggest a cortical (Shibasaki *et al.*, 1978; Hallett *et al.*, 1979; Obeso *et al.*, 1985), subcorticocortical (Thompson *et al.*, 1989) or reticular origin (Hallett *et al.*, 1977) for the myoclonic jerks. EEG and cortical SEP amplitudes were normal. Back-averaging of the EEG activity preceding the jerks did not reveal any cortical correlates. None of the patients exhibited any specific clinical signs of cerebral cortical or brainstem dysfunction. The duration of the EMG bursts responsible for the jerks was often very much longer than that seen in cortical (Hallett *et al.*, 1979; Obeso *et al.*, 1985), subcorticocortical (Thompson *et al.*, 1989) or brainstem reticular (Hallett *et al.*, 1977) myoclonus. Thus there was no evidence for a supraspinal origin for this myoclonus.

A spinal origin for the myoclonus

The features of all 3 cases are, however, consistent with a spinal origin of the jerks. In Case 1 the order of recruitment of muscles involved in the jerks was determined by the distance of their segmental supplies from T7, suggesting a myoclonic generator at about this level. In Case 2 jerks followed excision of a cervical haemangioblastoma and worsened with subsequent distortion of the cervical cord by dural adhesions seen on MRI and confirmed during surgical exploration. Jerks diminished following the division of the adhesions. The difference in latency between quadriceps and the middle portion of rectus abdominis in the stimulus-sensitive jerks (11.9 ms) was similar to that seen in the spontaneous jerks in Case 1 (13.0 ms). In contrast, the difference in latency between sternocleidomastoid and rectus abdominis (10.9 ms) was shorter than in Case 1 (44.1 ms), suggesting a more rostrally placed spinal myoclonic generator in Case 2. In Case 3 EMG activity was recorded first in the abdominal recti in both spontaneous and reflex jerks. The differences in latency between rectus abdominis and more rostral muscles were similar to those recorded in Case 1, and suggest a spinal origin for the myoclonus near the midthoracic cord. In addition there was a response in the left abdominal rectus to abdominal taps at a very short latency. The latter was incompatible with a transcortical reflex loop.

Hitherto, myoclonus of spinal origin has been described as focal (Halliday, 1967; Jankovic and Pardo, 1986), consisting of synchronous rhythmic jerks with a fairly constant frequency ranging from 20 to 180 per min, and confined to a group of muscles supplied by either one segment or several contiguous segments of the spinal cord (Halliday, 1967). Bursts of EMG activity may be relatively long (Hopkins and Michael, 1974; Davis *et al.*, 1981). Various spinal pathologies may give rise to segmental myoclonus (Lhermitte, 1919; Ellis, 1920; Campbell and Garland, 1956; Garcin *et al.*, 1968; Hopkins and Michael, 1974; Davis *et al.*, 1981).

The myoclonus described here was quite different from simple spinal segmental myoclonus. The axial flexion jerks were nonrhythmic and involved the recruitment of muscles innervated by spinal segments from the upper cervical cord to the lower lumbosacral cord. We suggest that this form of myoclonus arose in propriospinal systems.

Propriospinal myoclonus

The relatively stereotyped pattern of muscle involvement seen in the 3 patients suggests that the generalized axial jerks were subserved in each case by a similar pathway. In Case 1 the estimated conduction velocity within the spinal cord was slow ($5.0-6.5\ \mathrm{m \cdot s^{-1}}$). The differences in relative latencies between rectus abdominis and quadriceps (11.8 ms) in the stimulus-induced jerks in Case 2, and between rectus abdominis and deltoid (30.2 ms) in the spontaneous jerks in Case 3, were long and also suggest a slow spinal conduction velocity. In contrast, the conduction velocity in spinal efferent pathways is rapid in those forms of myoclonus with a supraspinal origin (Hallett *et al.*, 1977, 1979; Obeso *et al.*, 1985; Thompson *et al.*, 1989).

The slow spinal conduction velocity and the large jitter in latencies suggests a slowly conducting polysynaptic pathway. It is our hypothesis that these jerks arose in one or more adjacent spinal segments and then spread up and down the cord to other segments via propriospinal pathways.

There has been relatively little work on the propriospinal system in man. However, Giok (1958), studying the pattern of fibre degeneration in cases of ventrolateral cordotomy, concluded that a descending spinospinal fibre system exists in the ventrolateral funiculus, and there is some electrophysiological evidence to suggest the existence of both short (Malmgren and Pierrot-Deseilligny, 1988*a*, *b*) and long (Kearney and Chan, 1979; Meinck and Piesiur-Strehlow, 1981) propriospinal pathways in man. Meinck and Piesiur-Strehlow (1981), investigating a long-loop reflex from the rostral trunk and arms to lumbosacral motoneurons in healthy subjects, found a decrease in latency when more caudal segments were stimulated, and proposed that these reflexes were mediated by directly descending propriospinal pathways in man. In their study the difference in mean latency of the facilitation of the H reflex in quadriceps and gastrocnemius following ulnar nerve stimulation was 10 ms. Allowing for the shorter peripheral efferent time for quadriceps gives a 5 ms delay for central conduction from the L3/4 to the S1/2 segmental level, a distance of approximately 2.7 cm (Donaldson and Davis, 1903). The derived estimate of spinal conduction velocity in these propriospinal pathways in healthy subjects is slow ($5.4\ \mathrm{m \cdot s^{-1}}$) and compares favourably with the calculated conduction velocity in Case 1. The presence of inhibition at, or just preceding the onset of a jerk, as seen in the tonic EMG activity in Case 1, is also a feature shared by long propriospinal reflexes in man (Meinck and Piesiur-Strehlow, 1981).

Long propriospinal systems in the cat have been studied in more detail, and again bear close similarities to the generalized axial jerks. Long propriospinal pathways in the cat may originate in the cervical (Lloyd, 1942; Baldissera *et al.*, 1972; Jankowska *et al.*, 1974; Vasilenko, 1975) or midthoracic (Baldissera *et al.*, 1972) segments and project to lumbar segments, or they may originate in lumbar segments and project rostrally (Gernandt and Megirian, 1961; Barilari and Kuypers, 1969; Miller *et al.*, 1973). They are slowly conducting and may be polysynaptic (Lloyd, 1942; Vasilenko, 1975). In the 2 patients in whom jerks developed spontaneously, jerks were strikingly dependent on posture. In the cat, long cervical propriospinal neurons receive a very large vestibular input (Alstermark *et al.*, 1987*a*) as well as input from neck and limb afferents (Alstermark *et al.*, 1987*b*).

In all 3 cases the jerks involved axial and proximal limb muscles bilaterally, with cocontraction in antagonist and agonist muscle pairs. Similarly, the long propriospinal systems defined in the cat recruit mainly trunk (Baldissera *et al.*, 1972) and proximal limb (Vasilenko, 1975) motoneurons bilaterally (Barilari and Kuypers, 1969). Long propriospinal system s in the cat (Lloyd, 1942; Vasilenko, 1975) and man (Meinck and Piesiur-Strehlow, 1981) also share a lack of reciprocal action on antagonists. The functional importance of long spinospinal pathways principally innervating proximal muscle groups, without reciprocal organization, seems obscure until it is remembered that, in the cat at least, the spinal cord itself can generate forelimb locomotor movements coordinated with those of the hindlimbs (Miller and Van der Meché, 1976). Long propriospinal pathways are particularly suited to a role in interlimb coordination as the pattern of response generated by propriospinal projections varies systematically with limb position (Grillner and Rossignol, 1978) in static situations, and with the phase of the step cycle during locomotion (Forssberg *et al.*, 1977; Miller *et al.*, 1977).

Finally, an experimental model of propriospinally mediated myoclonus exists in the cat, where spinospinal pathways are thought to mediate the development of generalized rhythmic myoclonus following the inoculation of Newcastle disease virus into the lumbar, midthoracic or cervical cord, since the myoclonus in segments distal to the site of inoculation is abolished by thoracic spinal cord transection (Luttrell *et al.*, 1959). The myoclonus in this animal model persists after high cervical cord transection (Luttrell *et al.*, 1959).

Conclusion

Here we present 3 cases of axial jerking resulting in flexion at the neck, trunk, hips and knees, and suggest that the common clinical features of this new type of myoclonus are determined by the involvement of long propriospinal pathways in man.

It has always seemed likely that, because of the complex organization of its propriospinal systems, the spinal cord may give rise not only to simple focal segmental myoclonus, but also to a more generalized and complex form of myoclonus. Bussel *et al.* (1988) recently reported a patient with a cervical cord transection who developed rhythmic generalized extensor jerks, with relatively long EMG bursts, below the level of transection. Our 3 cases exhibited clinically similar generalized flexion jerks, again with relatively long EMG bursts in spontaneous jerks, and there was strong electrophysiological and pathological evidence for a spinal origin for the myoclonus in these patients. In addition to the 3 cases described here, and studied neurophysiologically, we have seen 3 other patients with clinically similar unexplained truncal flexor jerks, who may have a similar condition. Bressman and Fahn (1986) have also described 2 other patients (Cases 11, 12) whose clinical features are remarkably similar.

There is therefore an argument for expanding the present concept of spinal myoclonus to include two types: first, simple *spinal segmental myoclonus*, with focal repetitive rhythmic jerks confined to one or more adjacent spinal segments; and secondly, *propriospinal myoclonus*, with predominantly axial and often arhythmic flexor or extensor (Bussel *et al.*, 1988) jerks involving many spinal segments linked by long propriospinal pathways.

ACKNOWLEDGEMENTS

We thank Professor H. Shibasaki for his helpful comments, Mr R. Hayward and Dr I. N. F. McQueen for referring 2 of the patients, and Mr R. Bedlington for his technical assistance.

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(*Received October 11, 1989. Revised December 19, 1989. Accepted February 1, 1990*)

Brain (1991), **114**, 215–231

DUAL TASK PERFORMANCE AND PROCESSING RESOURCES IN NORMAL SUBJECTS AND PATIENTS WITH PARKINSON'S DISEASE

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SUMMARY

In recent years, there has been a growing consensus among investigators that the presence or absence of external cues guiding behaviour and attention is an important factor in determining whether or not deficits are found in patients with Parkinson's disease (PD). In an earlier study, the authors suggested that the pattern of impaired and intact performance could be explained in terms of differential resource demands of the tasks, combined with depleted levels of central processing resources in PD patients. Two experiments are reported, both employing dual-task paradigms. The first assessed, in normal subjects, the relative processing demands of a cued and an uncued version of the Stroop task. The results supported the proposal that the noncued task made greater demands on the subject's limited processing resources Further, performing a resource demanding secondary task concurrently with the Stroop test produced, in normal subjects, the same pattern of impaired performance as that reported previously in PD patients. In the second experiment the same dual-task paradigm was employed with a group of PD patients and normal aged-matched controls. Only the patients showed an increase in reaction time on the Stroop task when performing a resource demanding secondary task The patients also showed an interfering effect with concurrent foot tapping but not with an articulatory suppression task. The results were taken to support the hypothesis that PD patients have depleted central processing resources. In considering the present data, alternative explanations for the results are considered, in particular the possibility that they represent a deficit in switching processing resources between two tasks as the combined demands outweigh available resources.

INTRODUCTION

Brown and Marsden (1988*a*) suggested that patients with Parkinson's disease (PD) are impaired on cognitive tasks requiring internal cues but are relatively unimpaired when external cues are provided. This proposal is similar to those made by other authors in recent years. In explaining the pattern of impaired performance on recall and relative preservation of recognition memory, Weingartner *et al.* (1984) distinguished between *effort demanding* and *automatic* tasks. Flowers *et al.* (1984) suggested that 'In contrast to recall and learning tests, recognition is a 'passive' memory situation. Subjects do not have to manipulate the material, nor mentally 'scan' it ... nor do they have to organize actively a response ... A simple decision between fixed alternatives is all that is required.' Other authors have argued along similar lines, for tasks outside the domain of memory. For instance, Cools *et al.* (1984) argued that a deficit in 'set shifting' is found in 'behaviour not directed by currently available sensory information'. Taylor *et al.* (1986), in describing

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a pattern of impaired and intact performance on a wide range of tests, suggested that impairment was found on those tasks which required the 'spontaneous generation of task specific planning'. In the same paper, in relation to the pattern of performance on a series of memory tasks, the authors state that 'the presence or absence of impaired performance ... appeared to hinge on the degree to which internally, that is, subjectively organized guidelines are required for success. Patients performed adequately on the vast majority of memory tests where material was organized at presentation or presented in a recognition format.' Most recently, Ransmayr *et al.* (1987) observed a dissociation in performance on two visuospatial tasks. The authors noted that in the impaired task 'responses have to be conceived' while in the other, unimpaired, task 'responses have to be selected from several possibilities'.

There seems to be a growing consensus about the essential characteristics of the cognitive tasks which determine whether or not an impairment will be shown in PD patients. In an attempt to test the predictive value of the internal/external distinction, Brown and Marsden (1988*b*) employed a simplified version of the Stroop test (Stroop, 1935) in which the word RED was presented in green, and the work GREEN presented in red. For alternating blocks of 10 trials each, the subjects first had to identify the meaning of the word, then the colour in which it was presented, then the meaning of the word, and so on. In one condition (noncued), each stimulus was preceded by a warning signal READY. Subjects thus had to rely on their own 'internal' information to focus attention on the currently relevant stimulus attribute. In the other condition (cued), each stimulus was preceded by an explicit, 'external' cue, INK or WORD. In both patients and controls there was a marked increase in reaction time and error rate on trials where the subjects had to switch between the two stimulus attributes. This was then followed by an improvement in performance on subsequent trials until the next 'switch'. Control subjects showed no difference in performance between the cued and noncued conditions. PD patients, in contrast, were impaired on the noncued but not the cued conditions. Further, the impairment in the noncued condition was greatest for the trials where the subject had to switch between the two attributes.

These results support the internal/external distinction, at least as a description of the situations in which deficits are found in PD patients. However, describing the nature of the tasks which are impaired is not the same as describing the nature of the processes involved. To progress beyond simple description, a theoretical framework is needed which can explain the observed data and allow predictions to be made. As has been discussed, the internal/external distinction can be applied to a wide range of cognitive tasks, e.g., memory, visuospatial function, attention. Rather than propose individual theories for each domain of cognitive function, it is preferable, at least initially, to attempt to find a general framework for the data. The putative deficit, therefore, must be at some stage of processing, or involve some component, common to a wide range of tasks.

The Working Memory model (Baddeley *et al.*, 1986) provides a possible framework. When we perform *any* cognitive task, we need to access and process information from the current environment together with the information contained in long-term store. Obviously an interface is required for those different types of information. This interface, which has been labelled Working Memory, is seen as being divided into a Central Executive and a number of Slave Systems. These latter systems include an Articulatory Loop which permits the recycling of verbal information while it is being processed, and a Visuospatial Sketchpad which permits the temporary storage and manipulation of visuospatial information. The Central Executive is seen as acting as a supervisor or scheduler, selecting strategies and integrating information from several different sources. Its precise nature and characteristics, however, have not been elaborated to the same degree of those of the slave systems. Instead, Baddeley *et al.* (1986) adopted the attentional model of Norman and Shallice (1980) and applied it to the Central Executive. Norman and Shallice proposed a model of attentional control within the framework of action. Many actions, such as walking, are automatic. Sets of motor programs controlling individual muscles form higher order motor plans or schemata which can be executed without conscious effort. Several different schemata or plans may run in parallel allowing us to perform different tasks simultaneously. There will be times, however, when the different schemata will conflict. The model proposes an automatic processor or 'Contention Scheduler' which gives precedence to one of the conflicting schemata at any one moment on the basis of preset priorities and environmental contingencies. However, we can override automatic action and consciously focus our attention elsewhere. Norman and Shallice (1980) proposed a 'Supervisory Attentional System' (SAS) to serve this function. The SAS is assumed to have limited capacity and is therefore called upon only under certain situations. These include (1) planning and decision making, (2) novel or poorly learned tasks and where preprogrammed schemata are not available, and (3) situations where some strong habitual response or temptation is involved.

Tasks such as the Stroop task can place a large attentional demand on the subject. The subject must select one of the two stimulus attributes, while attempting to inhibit the other. In the version used by Brown and Marsden (1988*b*), the demands were greater still as the relevant attribute was not constant. Such a task might therefore be expected to rely heavily upon the SAS. In order for the SAS to work efficiently it needs information on the current status of the task. The results from Brown and Marsden (1988*b*) suggested that when the information was provided from an external source, the SAS of PD patients was able to function relatively normally. When it had to rely on internally generated information, however, more errors were committed and processing time increased. Two main possibilities exist to explain this pattern within our framework. The first relates to the nature of the information which the SAS uses, while the second relates to the characteristics of the SAS itself.

To consider the first option, it is possible that the internal information controlling attention is stored in a degraded or disorganized way. The result of this might be slower and less accurate retrieval of that information by SAS from short-term memory (STM). Evidence for a STM deficit in PD is, however, equivocal. In the majority of studies, immediate digit span has been unimpaired (e.g., Asso, 1969; Horn, 1974; Riklan *et al.*, 1976; Lees and Smith, 1983; Hietanen and Teräväinen, 1986). An experiment by Tweedy *et al.* (1982), however, assessed the recall of work triads using the Brown-Peterson short-term memory paradigm (Peterson and Peterson, 1959). While the patients' immediate recall was normal, they showed impaired performance after intervals of only 3 – 18 s when the delay was filled with a task to prevent rehearsal. This finding, however, is of doubtful significance for the experiment of Brown and Marsden (1988*b*), as the subjects were free to employ rehearsal as a strategy to maintain the cue in a salient and usable form.

The remaining possible explanations lie with the SAS itself. While the information may be available, the SAS may have difficulty in accessing and/or utilizing it to control attentional resources. A utilization deficit is unattractive, however, as it requires that we postulate different mechanisms in the SAS for dealing with information from different sources, internal or external. A deficit in accessing the internal information is more appealing. Presumably different pathways are involved in accessing stored (internal) information and that coming via cortical association areas (external information). In PD patients there may be a dysfunction in this internal information route to the SAS, while the external information route may be normal. While it is a viable model, caution is necessary in proceeding further at this stage. Norman and Bobrow (1975) suggested that in general, a dissociation between performance on two tasks does not necessarily imply differences in the functioning of two systems or subsystems. It is possible that the results derive merely from a quantitative difference in the resource requirements of the two tasks. Based on this argument, Brown and Marsden (1988*b*) suggested that internally cued tasks are more resource demanding and that PD patients have fewer processing resources to allocate to SAS.

Norman and Bobrow (1975) introduced a conceptual scheme for describing the relationship between resources and performance, the Performance Resource Function or PRF. In all tasks there is a minimum level of resource allocation (R_{min}) needed before an output can be produced. In many tasks there then follows a phase in which performance increases as more resources are allocated. This is known as the 'resource limited' phase. The exact shape of the function relating resources to performance will probably vary from task to task. A point (P_{max}) will be reached in most tasks, however, where increasing resources have no effect on performance. At this point, the performance is said to have become 'data limited'. Factors such as signal quality, amplitude, presentation rate, etc., as well as the quality of memory representations, will influence performance on the task, no matter how much resource the subject allocates up to the resource limit (L). It may be assumed that the most 'efficient' resource allocation will maintain performance close to the point where it starts to become data limited (R_{dl}).

Returning to the results of Brown and Marsden (1988*b*), how may their data be interpreted within this scheme? Switching attention between stimulus attributes would seem likely to draw heavily on the SAS. External cues could be seen as reducing these demands. The cued and noncued Stroop tasks may thus have different resource requirements. Fig. 1 shows hypothetical PRFs for the cued and noncued Stroop task, each with a resource limited and data limited phase. The slope of the noncued task is such that more resources need to be allocated to achieve the same level of performance as for the cued task. Provided, however, the points R_{dl} for both tasks are less than the available resources, performance on the two tasks will be the same. This can account for the equivalent performance of the control group on the two tasks, despite the assumption that they make different demands on the SAS. What, however, if the amount of available resource is reduced? Provided the level is still greater than R_{dl} for both tasks, there will be no impact on performance. This is indicated by point r_1. If resources are reduced to point r_2, performance on the cued task, still in the data limited phase, will be unaffected. The more resource-demanding noncued task, however, is now in the resource limited phase, and performance reduces to the level indicated by $P_{2(noncued)}$. This is the pattern of performance observed in the PD group. Finally, what

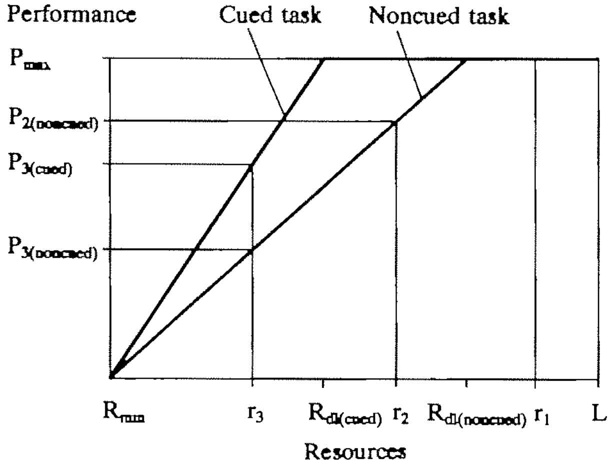

FIG 1. Hypothetical Performance Resource Functions for the cued and noncued Stroop tasks. The x axis represents the level of resources from the upper limit (L) to the functional minimum (R_{min}) The points at which performance on the tasks changes from being performance limited to data limited is indicated by the points $R_{dl(cued)}$ and $R_{dl(noncued)}$. Various performance levels for the cued and noncued task are indicated on the y axis, corresponding to different resource levels

if resources are reduced further to point r_3? Now both cued and noncued tasks are in their resource limited phases and both will show levels of performance less than P_{max}. Because of the slopes of the PRFs, however, performance on the cued task will always be superior to the noncued task, down to the limit R_{min}. The model of depleted processing resources in the SAS of PD patients seems to fit the results from the experiment of Brown and Marsden (1988*b*) reasonably well. This application of the resource model, however, is post hoc and depends upon the critical assumption that the cued and noncued version of the Stroop task made different demands on the limited processing resources of the SAS. Before proceeding with further experiments on patients with PD, it was necessary to test this assumption using a group of normal control subjects.

A method for assessing task processing demands is provided by use of a dual-task paradigm. A resource demanding secondary task is performed concurrently with the task of interest, the primary task. The concept of a central limited capacity processor such as the SAS implies that if the combined processing demands of the two tasks exceed the available resources, then performance on one or both of the tasks will deteriorate, depending on the strategy employed by the subject or the requirements of the task. The usual aim is to choose a relatively simple, if resource demanding, secondary task which can be performed to a high level of accuracy, and to measure the impact on the primary task. One such task is random number generation. In order to monitor performance, it is necessary for the subject to vocalize the random numbers which introduces an additional factor. If performance on the primary task is dependent upon verbal rehearsal or problem-solving strategies, then the simple act of articulating the secondary task may interfere with the primary task, independent of its resource demanding characteristics. Because of this, it is usual for an additional condition to be included in which the subject performs the primary task concurrently with a (presumed) automatic secondary task which involves the same articulatory component as the random generation or digit span task. Typically the subject is asked to repeat a single word or syllable.

If the hypothetical PRFs in fig. 1 represent the resource demands of the cued and noncued Stroop tasks, then the effect of a resource-demanding secondary task should be to reduce the level of resources available for the Stroop task. Even in normal subjects, if this level is less than the point $R_{dl(noncued)}$, performance on the noncued task should fall below that of the cued task, just as it does in patients with PD.

EXPERIMENT 1

Methods

Subjects

Forty volunteer subjects (20 male, 20 female) were chosen between the ages of 20 and 40 yrs (mean age 29.8 yrs, SD 4.1). All had been educated to at least graduate level. None had a history of head injury or neurological disorder, and none were taking minor tranquillizers or antidepressants. Subjects were randomly assigned to one of four groups (*see below*), with the one constraint that each group contained equal numbers of males and females. The mean ages of the subjects in the four groups were, respectively, 29.5 yrs (SD 4.1), 28.8 yrs (SD 4.2), 29.8 yrs (SD 4.3) and 31 3 yrs (SD 3.8). The mean ages of the four groups did not differ statistically ($F(3,36) = 0.63$, $P>0.05$). All subjects gave informed consent.

Primary tasks

The two primary tasks were adapted versions of the Stroop task described previously (Brown and Marsden, 1988*b*). Each condition of the task comprised 80 trials, made up of a random series of the 2 Stroop stimuli, RED presented in 2 cm high green letters, and GREEN presented in red letters The relevant attribute to which the subject had to respond changed every trial from 'Ink' (the colour in which the word was presented) to 'Word' (the meaning of the word) to 'Ink' and so on throughout the 80 trials. The subjects were told that the first trial was always an 'Ink' trial. The 2 primary tasks differed in that in one condition ('cued'), the subject received the explicit cue INK or WORD on the VDU screen immediately prior to the Stroop stimulus, while in the other condition ('noncued'), the subject received only the warning signal READY. The cue or warning signal was presented for 1 s before being replaced, at the same screen location, by the test stimulus. This remained present for 1 s after the subject had made a response. Errors were signalled by a tone. No feedback was given for correct responses After a delay of 1.5 s the next cue or warning signal appeared. All subjects received both versions of the task. Half received the cued condition first, and half the noncued.

The 80 trials of each condition were divided into 5 blocks of 16 trials each. At the end of the first block, the instruction STOP appeared on the VDU screen. This was followed, after a 2 s delay, by the instruction to begin the secondary task (with the exception of group 1; *see below*). After 2 s the primary task continued with the first 'ink' trial. The subjects had to continue performing the secondary task for the next 16 trials, after which they were again given the instruction STOP. The next block of 16 trials was then performed without a secondary task. This was followed by 1 more block of trials with the secondary task and then a final block without. Thus there were 2 blocks (2 and 4) with a secondary task, and 3 without (1, 3 and 5). Mean reaction times were calculated for the nonerror trials for each block of 16 trials. Total errors were calculated for each of the 5 blocks within each condition.

The response apparatus was a box with 1 inch red and green buttons placed 9 inches apart. Subjects sat holding the box such that their thumbs rested on the two buttons. The experiment was controlled by a BBC Master Series microcomputer, fitted with a 65C102 internal coprocessor. Reaction times were measured to the nearest 0.01 s. The stimuli were presented on a 14 inch Microvitec high resolution colour monitor.

Secondary tasks

Random number generation was chosen as the resource demanding secondary task. An articulatory suppression condition was included to assess the effects of vocalization on the primary task. Finally, foot tapping was included as a third secondary task to assess the possible interfering effect of a repetitive motor response, a feature common to the two other secondary tasks, but without any vocalization.

Group 1—No secondary task. Subjects in group 1 served as controls and had no secondary task to perform in blocks 2 and 4.

Group 2—Tapping. For group 2, the secondary task was to tap the right foot on the floor at a rate of approximately 2 Hz. Each subject practised the secondary task on its own before commencing the main experiment. The investigator was present throughout the experiment to ensure that the subject performed the secondary task adequately when required. If the tapping rate was judged to be too fast or too slow, the subject was given a predetermined visual signal by the investigator to modify the rate.

Group 3—Articulatory suppression. For group 3, the secondary task was to repeat the word 'blah' at a rate of approximately 2 Hz. As with the tapping task, each subject was trained before-hand, and was given feed-back during the task if the rate altered.

Group 4—Random number generation. For group 4, the secondary task was to produce 'random' single digit numbers between 1 and 9, at the rate of approximately 2 Hz. As with the other tasks each subject received training before commencing the main task. In addition, a baseline measure of the subject's 'randomness' was taken by asking him or her to produce a series of 120 numbers which were recorded for subsequent analysis (*see below*). The random numbers generated in blocks 2 and 4 of the main task were likewise recorded.

The turning point index of randomness was used (Kendall, 1973). A turning point was defined as a number which was either greater than its two neighbouring values in the series (e.g., 2-5-4) or less than its two neighbouring values (e.g., 6-3-8). Thus the series 1-4-6-3-1-7-5 has three turning points, 6, 1 and 7. In a fully random series of size n, the expected number of turning points is $2(n-2)/3$. In assessing 'randomness' an index was taken, on each occasion, of the percentage difference between the expected and actual number of turning points.

Results

Group 4—Random number generation

Before considering differences in the four groups on the primary task, it is necessary to examine the performance of group 4 on the secondary task, generation of random numbers. A crucial question to answer is whether there was any difference between the subjects' performances in generating random numbers in the cued and noncued conditions. If performances on the two primary task conditions are to be compared directly, it is necessary to demonstrate that the subjects were producing equally random series in the two conditions and, by inference, allocating equal resources to the secondary task. The mean difference score for blocks 2 and 4 was 11.5% (SD 9.32) for the cued condition and 8.9% (SD 6.8) for the noncued condition ($t = 1.46$, $df = 9$, $P > 0.05$). There was no evidence, therefore, that the mean level of randomness was significantly different in the cued and noncued conditions.

Group comparison on the primary tasks

Reaction time data. Fig. 2 shows the mean reaction times for the 5 blocks in the cued and noncued conditions, respectively, in each of the four groups of subjects. Repeated measures analysis of variance using MANOVA (SPSS, 1988) revealed significant main effects for Group (1–4) ($F(3,36) = 5.14$, $P < 0.05$) and for Block (1–5) ($F(4,33) = 8.23$, $P < 0.05$), but not for Condition (cued/noncued) ($F(1,36) = 1.07$, $P > 0.05$). All of the two-way interactions were significant (Condition×Block ($F(4,33) = 7.26$, $P < 0.05$), Group×Condition ($F(3,36) = 3.55$, $P < 0.05$) and Group×Block ($F(12,105) = 2.70$, $P < 0.05$)). The three-way interaction Group×Block×Condition was also significant ($F(12,105) = 3.55$, $P < 0.05$).

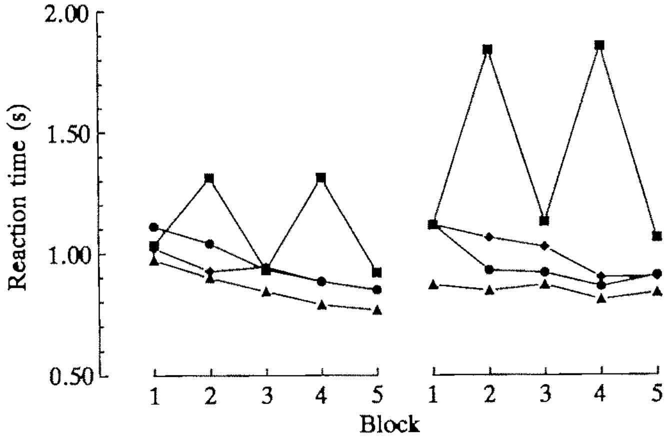

FIG. 2 Experiment 1. Mean reaction time (s) for the 5 blocks of the cued and noncued Stroop tasks for each of the four groups of subjects Group 1, no secondary task (▲); Group 2, tapping (●), Group 3, articulatory suppression (◆), Group 4, random number generation (■).

To aid in interpreting these various findings, a series of post hoc comparisons were made. First the effects of Block, Condition and the Block×Condition interaction were assessed in each group in turn. The effect of Block, averaging across the two conditions failed to reach significance for any of the four groups, although it approached significance in all cases (group 1 (no secondary task) (F(4,6) = 3.41, $P < 0.10$), group 2 (tapping) (F(4,6) = 4.28, $P < 0.10$), group 3 ('blah') (F(4,6) = 3.42, $P < 0.10$), group 4 (random numbers) (F(4,6) = 3.57, $P < 0.10$)).

The effect of Condition (cued/noncued) averaging across blocks was clearly nonsignificant for group 1 (F(1,9) = 0.19, $P > 0.05$), group 2 (F(1,9) = 0.62, $P > 0.05$) and group 3 (F(1,9) = 0.08, $P > 0.05$). In contrast, a significant effect of condition was observed for group 4, the random number group (F(1,9) = 8.55, $P < 0.05$). The Block×Condition effect was nonsignificant for group 1 (F(4,6) = 1.78, $P > 0.05$) and group 2 (F(4,6) = 2.15, $P > 0.05$), but significant for group 3 ('blah') (F(4,6) = 3.68, $P < 0.05$) and group 4 (random numbers) (F(4,6) = 24.19, $P < 0.05$).

The next comparisons were a series of one-way analyses of variance, comparing the four groups for each block in turn for the cued and noncued conditions. For the cued condition, no significant difference was found between the groups for block 1 (F(3,36) = 0.34, $P > 0.05$), block 3 (F(3,36) = 0.43, $P > 0.05$) or block 5 (F(3,36) = 0.65, $P > 0.05$), the blocks performed *without* a secondary task. Significant omnibus F statistics were found, however, for block 2 (F(3,36) = 4.66, $P < 0.05$) and block 4 (F(3,36) = 5.08, $P < 0.05$), the blocks performed *with* a secondary task. Paired comparisons between the groups using the Newmann-Keuls test revealed that groups 1 and 2 differed from group 4 (random numbers) for block 2, and groups 1 and 3 differed from group 4 for block 4. A very similar picture was found for the noncued tasks, except that the differences between the groups in block 2 (F(3,36) = 12.57, $P < 0.05$) and block 4 (F(3,36) = 16.08, $P < 0.05$) appear even more striking. Paired comparisons revealed that in both cases groups 1, 2 and 3 differed from group 4 in both blocks performed with a secondary task. In contrast, no group difference was demonstrated for either block 1 (F(3,36) = 1.71, $P > 0.05$), block 3 (F(3,36) = 1.03, $P > 0.05$) or block 5 (F(3,36) = 1.25, $P > 0.05$).

The statistics so far confirm the pattern of results suggested by fig. 2. The most striking feature is the separation of group 4 from the remaining groups on the two blocks with secondary tasks. In contrast, the remaining three groups do not differ in the blocks either with or without secondary tasks. The final set of analyses concern only group 4, the random number group. A series of paired t tests were conducted to compare the group's performance between the cued and noncued conditions for each of the 5 blocks. No significant difference was found for block 3 ($t = 1.45$, df = 9, $P > 0.05$) or block 5 ($t = 1.39$, df = 9, $P > 0.05$), while for block 1, the difference approached significance ($t = 1.87$, df = 9, $P < 0.10$). Significant effects were found, however, for block 2 ($t = 3.04$, df = 9, $P < 0.05$) and block 4 ($t = 3.10$, df = 9, $P < 0.05$). Thus, within group 4, there was a greater effect of the secondary task on Stroop performance in the noncued conditions compared with the cued condition.

Error data. The number of errors made by subjects in some of the groups was extremely low, with many subjects making no errors. Median errors for the four groups are shown in fig. 3. Details of nonparametric analysis will not be given as the results essentially parallel those of the reaction time data. Random number generation was the only secondary task to increase error rate, with a greater increase in the noncued condition.

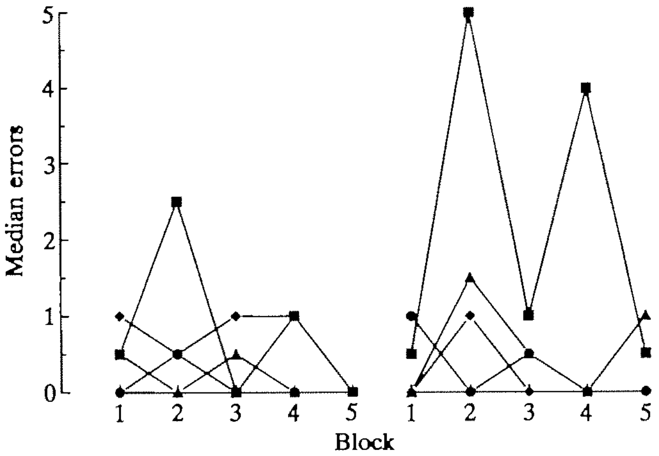

FIG. 3. Experiment 1. Median errors for the 5 blocks of the cued and noncued Stroop tasks for each of the four groups of subjects. Symbols as in fig. 2.

Discussion

The aim of the first experiment was to investigate the relative processing demands of a cued and noncued Stroop task. Brown and Marsden (1988*b*) proposed that the noncued task made greater demands on the limited capacity central processor the SAS.

A difference in performance, however, would not be apparent if the demands of both tasks were within the available processing resources. It was predicted that giving the subject an additional resource demanding task, in this case random number generation, should overload the SAS leading to a deterioration in performance on the primary task. The degree of deterioration, however, would depend on the degree to which the SAS was overloaded. If the cued and noncued tasks differed in their processing requirements, as proposed, then the random generation task should have a more marked effect on the noncued condition.

The results of the experiment largely confirmed this prediction. For both the cued and noncued conditions, random number generation lead to a significant increase in reaction times and error rate on the primary task. As neither foot tapping nor articulatory suppression had any significant effect on the Stroop task, for either condition, the influence of random generation could be explained in terms of resource demands of the task and not its motor or verbal components. As both the cued and noncued Stroop tasks were effected by random generation, it implies that both conditions depended on the functioning of a limited central capacity processor. As predicted, however, the effect was greatest with the noncued Stroop condition, implying that the processing demands of that task were greater than those of the cued condition. Thus removing some of the available resources using the random generation task produced, in the normal controls, a pattern of performance similar to that found previously in patients with PD. While this supports the hypothesis that patients with PD have fewer resources in the SAS than age-matched controls, the support is only indirect. It is still necessary to demonstrate that the performance of patients with PD on a task such as the Stroop, can be differentially affected by manipulating their available processing resources. This was the purpose of the next experiment.

EXPERIMENT 2

Methods

Subjects

Parkinson's disease group. Eighteen patients with idiopathic PD were assessed. The diagnostic criteria were those described by Quinn and Husain (1986). After data collection, however, the diagnosis of PD for 1 of the patients was called into question in the light of new clinical information. This patient was excluded from the subsequent analyses.

The patient group comprised 11 males and 6 females with a mean age of 65.2 yrs (SD 6.4). Mean approximate age of onset of PD was 55.6 yrs (SD 8.1), with a mean duration of illness of 11.6 yrs (SD 4.7). All subjects had bilateral disease, either Hoehn and Yahr stage II (12 subjects) or III (5 subjects). All patients were taking levodopa (mean dose 641 mg/day, SD 214) in conjunction with a dopa-decarboxylase inhibitor. In addition, 2 subjects were taking amantadine, and 6 were taking anticholinergics, either benzhexol or orphenedrine.

Control group. Eighteen control subjects were assessed. The data from 1 subject, however, were excluded. This was the subject matched for order of testing with the excluded patient. The control subjects were recruited from a volunteer subject panel run by the University of Manchester Age and Cognitive Performance Research Centre. The same exclusion criteria were applied as for Experiment 1. The control group comprised 8 males and 9 females with a mean age of 66.5 yrs (SD 4.9).

Control variables and motor measures

Each subject was assessed on a set of visual-analogue scales measuring affect and arousal state (Bond and Lader, 1974). In addition, they received the Vocabulary subscale of the WAIS (Wechsler, 1955).

Experimental tasks

The second experiment employed the same alternating Stroop paradigm developed for Experiment 1. Certain modifications, however, were necessary before using it with an ageing clinical population. Several subjects in the first experiment reported considerable difficulty coping with the noncued Stroop condition while generating random numbers. Accordingly it was decided that the noncued Stroop condition would prove too difficult for an ageing sample, both patients and controls. The purpose of the experiment was not to assess the relative resource demands of the cued versus the noncued Stroop tasks, but the resource levels of the subjects. From the previous experiments, it would seem that the cued and noncued Stroop tasks occupy points on a continuum of resource demand. Use of the cued task, although less resource demanding, would still permit an assessment of the relative resource levels in the two groups. It was decided, therefore, to use only the cued condition in the present experiment. A further change from Experiment 1 was the slowing of secondary task performance from 2 items to 1 item per second. This decision, again, was taken in the light of feed-back from subjects in Experiment 1.

It was predicted that the tapping and articulatory suppression tasks would have little effect on primary task performance in either the PD, or in the control groups. Random number generation, however, should produce a small but significant effect in the control group, and a larger decrement in performance in the PD group.

The same apparatus and mode of response was used as for the last experiment. Standardized instructions, examples and practice items were presented via the VDU before commencing each task. Data collection for each task lasted 10–15 min. Subjects received a break in between each task. The Stroop task was similar to that for Experiment 1. A sequence of Stroop stimuli was presented, and the subject had to respond, alternately, to the colour of the 'ink' and the meaning of the word. The subject received an explicit cue, INK or WORD, before each stimulus. Each condition comprised 40 trials, made up of a random series of the 2 Stroop stimuli. The subjects were told that the first trial was always an 'ink' trial. The 40 trials of each condition were divided into 4 blocks of 10 trials each. The end of each block was signalled by the instruction STOP appearing on the VDU screen, followed by a break of 20 s during which the subject rested. The end of the period was signalled by a 1 s tone, and followed 1 s later by the first cue or warning signal. The interstimulus intervals were the same as for Experiment 1. As before, errors were signalled by a tone, but no feed-back was given for correct responses. Mean reaction times were calculated for the nonerror trials for each block of 10 trials. Total errors were also calculated for each of the 4 blocks within each condition.

Unlike in Experiment 1, a mixed between group/within subject design was used, with each subject performing the cued Stroop task with each of the 3 secondary tasks in a balanced order. At the beginning of each block of 10 trials the subjects received the command to begin the secondary task. This was then performed until the 10 Stroop trials of the block had been completed and the instruction STOP given Each secondary task was performed for 4 blocks of 10 trials.

The same 3 secondary tasks were used as for Experiment 1, tapping, articulatory suppression and random number generation, with a planned rate of approximately 1 Hz. Immediately before performing a Stroop task with random number generation, the subject was required to generate 100 random numbers as the sole task. This served to familiarize with random number generation, and to provide a baseline measure of their 'randomness'. As in Experiment 1, the random numbers generated by each subject were analysed using the index of turning points. The experimenter was present throughout testing to ensure that the subject performed the secondary tasks as instructed.

Results

Age, education, cognitive and motor function and affect-arousal

The Table shows the mean ages, years of education, WAIS Vocabulary and the affect-arousal scores of the two groups. There was no difference between the mean ages of the two groups ($t = 0.66$, $df = 32$, $P > 0.05$), their number of years of education ($t = 0.31$, $df = 25.59$, $P > 0.05$), their WAIS Vocabulary scores ($t = 0.99$, $df = 32$, $P > 0.05$), or their ratings of affect-arousal ($t = 1.12$, $df = 32$, $P > 0.05$).

TABLE DESCRIPTIVE STATISTICS FOR THE PARKINSON'S DISEASE AND CONTROL GROUPS

	Patient group (n=17)		*Control group (n=17)*	
	Mean	*(SD)*	***Mean***	*(SD)*
Age (yrs)	65 2	(6.3)	66 4	(4 9)
Education (yrs)	11 8	(3 4)	11.5	(1.9)
WAIS Vocabulary	13.5	(2 9)	14.1	(2.2)
Subjective affect-arousal	30.9	(11.7)	25.1	(14 8)

Random number generation

While both patients and controls were able to produce numbers at the required rate of 1/s during the baseline test, they were both noticeably slower at producing random numbers during performance of the Stroop task. Averaging across all 4 blocks, the mean rate of production was 0.64 numbers/s (SD 0.25) for the patients, and 0.69 (SD 0.25) for the controls. The mean rate, however, did not differ for the two groups ($t = 0.72$, $df = 32$, $P > 0.05$).

For the baseline series the patients produced 22.3% (SD 18.3) fewer turning points than expected, while the controls produced only 8.7% (SD 8.9) fewer. The same pattern was seen with the overall random numbers generated across the four blocks of the Stroop task (26.2% (SD 11.9) for the patients and 17.8% (SD 9.9) for the controls). Repeated measures ANOVA revealed a significant difference between the groups averaging across the baseline and Stroop testing ($F(1,32) = 11.8$, $P < 0.05$), but no significant interaction between the groups and occasion of testing ($F(1,32) = 0.75$, $P > 0.05$). The main effect for occasion, averaging across the two groups, was significant ($F(1,32) = 4.70$, $P < 0.05$). Thus while the patients' random number generation was less satisfactory than that of the controls, both groups produced fewer turning points when generating random numbers at the same time as performing the Stroop. The deterioration in performance, however, was the same in both groups.

Primary task—reaction time data

Fig. 4 shows the mean reaction times (averaged across blocks) of the two groups for the cued Stroop task during performance of each of the 3 secondary tasks. Repeated measures ANOVA revealed a significant main effect for Task (tapping, articulatory suppression or random generation) ($F(2,31) = 7.60$, $P < 0.05$) and for Group ($F(1,32) = 3.78$, $P > 0.05$). The Group×Task interaction approached significance ($F(2,31) = 2.58$, $P < 0.10$) with the multivariate test, but was significant using the more powerful univariate approach, even after adjusting the degrees of freedom to correct for sphericity ($F(1.5,43.6) = 4.22$, $P < 0.05$). The interaction was investigated further with a series of post hoc analyses. First, the secondary task effect was assessed for each group independently. A significant main effect for task was found for the PD group ($F(2,31) = 9.64$, $P < 0.05$), but not for the control group ($F(2,31) = 0.69$, $P > 0.05$). Within the patient group, tapping produced more interference than articulatory suppression ($t = 2.75$, $df = 16$, $P < 0.05$), while random number generation produced more interference than the mean of the other 2 secondary tasks ($t = 3.21$, $df = 16$, $P < 0.05$).

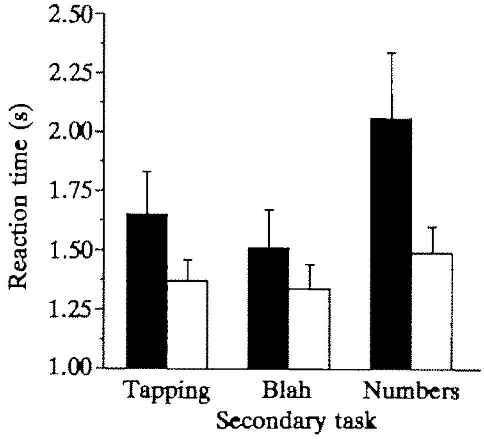

FIG 4. Experiment 2. Mean (+SEM) reaction time (s) on the cued Stroop task for the Parkinson's disease (closed columns) and control groups (open columns), under each of the 3 secondary task conditions

Primary task—Error data

Error rates were generally higher than in Experiment 1. With square root transformation, the data were considered suitable for parametric analysis. Fig. 5 shows the square root transformed data for the two groups under the 3 secondary task conditions. The effect of Condition was significant (F(2,31) = 12.69, $P < 0.05$), but not the main effect of Group (F(1,32) = 2.32, $P > 0.05$) or the Group×Condition interaction (F(2,31) = 0.84, $P > 0.05$). No further analyses were performed on these data.

To summarize, the patients with PD showed a significant increase in reaction time when performing the cued Stroop task with random number generation. A slowing of reaction time with tapping as compared with articulatory suppression was also seen in

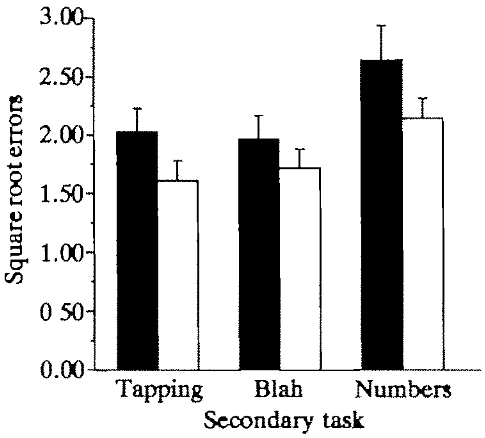

FIG 5. Experiment 2. Mean square root (+SEM) errors on the cued Stroop task for the Parkinson's disease and control groups, under each of the 3 secondary task conditions. Symbols as in fig. 4.

the PD group but not in the controls. For the error data, both groups showed an increase in errors in the random generation condition. The pattern of performance, however, was the same in the two groups.

DISCUSSION

The aim of the second experiment was to compare, using a dual task paradigm, the relative central processing resources in patients with PD and normal control subjects. The primary task employed was the cued version of the Stroop test. The crucial test was the difference between the effects on this primary task of the resource demanding random generation task, compared with the tapping and articulatory suppression tasks, presumed to be less or not resource demanding.

In the control group there was no significant difference between the reaction time for the primary task under the 3 secondary task conditions. This latter result implies that the combined demands of the cued Stroop task and the various secondary tasks were within the available processing resources of the control subjects. In contrast, the additional load imposed by the random generation task led to a deterioration in the reaction time performance in the PD group. A parsimonious conclusion from this finding, in line with the initial hypothesis, is that the patients with PD had fewer processing resources, and that the combined tasks overstretched those that were available.

One unpredicted result was the interfering effect of foot tapping on primary task reaction times in the PD group, when compared with the effect of articulatory suppression. The execution of simple repetitive motor acts is known to break down in patients with PD (Marsden, 1985). This is clearly visible in movements involving the limbs. In movements which would normally be performed automatically, a degree of conscious control may be needed or, in other words, the task may become resource demanding. In the context of the present experiment, foot tapping in the patients may have been resource demanding, whereas in the controls it would have been relatively automatic. This fits in with the finding of the interfering effect of foot tapping in the patient group. The articulatory suppression condition, however, had less of an effect on the primary task in the PD group, even though it also involved a repetitive motor action. This has implications for the mechanisms underlying the control of the two motor actions, and their functioning in patients with PD. It suggests, further, that dual task paradigms may provide a useful tool for examining the processes of motor control in movement disorders (*see* Heuer and Wing, 1984).

In Experiment 1, an interfering effect of random generation on reaction time was observed in the normal control subjects, even in the cued condition. No such effect was noted in the second experiment, despite the age of the control sample. One explanation for this finding may relate to the fact that the subjects in the second experiment received more exposure to the Stroop task than those in Experiment 1. In Experiment 2 one-third of subjects would have performed the Stroop task twice before performing the random generation condition, while a further third would have performed it once before. The cued Stroop task may, therefore, have had different resource demands, on average, in the two experiments. These may have been sufficient to show an effect of random number generation in the patient group but not in the controls. The rate of random number production in the two experiments may also have contributed to the difference in findings.

In Experiment 1, the rate was 2 Hz. In Experiment 2 this was reduced to 1 Hz, and the actual rate achieved was even slower at 0.64–0.69 Hz. Again, this may have reduced the resource demands of the random number generation to the extent that they were insufficient to overtax the resources of the controls. Only in the patients, whose resource levels were already compromised, would the reduction in the level of available resources have an impact on primary task performance.

These results are consistent with the resource depletion hypothesis being tested. The same model can accommodate the findings of earlier experiments which showed dissociations between tasks involving internal and external cues, and the results of the present dual task paradigm which produced an impairment in PD even in an externally cued task. It is important, however, to consider whether alternative or additional explanations can be found for the present findings.

The first possibility is not that the patients with PD have fewer resources to allocate, but that some tasks are more resource demanding for the patients than they are for the controls. If used as a secondary task, the result would be fewer resources to allocate to the primary task and impaired performance, even if the absolute level of resources were the same in both groups. Although perfectly feasible, this explanation is heuristically less attractive than the resource depletion hypothesis as it does not say *why* some tasks are more resource demanding for patients than for controls. In effect, we are saying nothing more than that some tasks are more difficult for patients with PD. Therefore, to move from a hypothesis based on a higher-order deficit to one based on task-specific deficit is unattractive, at least at present, until the limits of the resource model are further explored.

The second possibility is that even if the resource depletion hypothesis is valid, other additional factors may have contributed to the pattern of results in the present experiments. The control subjects in Experiment 1, but not those in Experiment 2, showed a decrement in Stroop performance when random number generation was the secondary task. While this can be accommodated within the resource depletion model as discussed above, an alternative explanation can be found in terms of time sharing between the two tasks. The slower rate of number generation in Experiment 2 would have made it possible for subjects to switch resources between the two tasks throughout the block of trials. Such a strategy would have been made easier by the presence of the Stroop cue which would signal, in advance, that a switch was necessary. What implications does this have for the deficit in the PD patients in Experiment 2? Rather than (or in addition to) fewer processing resources, the patients may have problems in the strategic allocation of those resources, switching them from task to task as required. Slowness in switching resources from number generation to the Stroop would have the effect of increasing reaction time on the Stroop without necessarily leading to an increase in the error rate, interestingly the pattern of results obtained. Although a switching hypothesis makes no assumptions about resource levels, it still retains the focus on the SAS, one of whose roles is the strategic allocation of the available resources.

Thus while a deficit in resource-switching may have less general explanatory power than a resource-depletion hypothesis, it may be of value in dual task experiments where task demands permit or necessitate the subject employing a time sharing strategy. Further experiments, investigating the precise timing characteristics of primary and secondary tasks performance in dual task paradigms, should help to determine whether a time sharing strategy is being employed and the degree to which it is impaired in patients with PD. In addition, the resource depletion hypothesis needs to be further assessed by the use of a wider range of primary and secondary tasks. Using secondary tasks in which resource demands can be manipulated will help to determine the characteristics of resource levels and allocation in patients with PD, while use of a range of secondary tasks will enable us to determine the degree of specificity of the dual task decrement.

In conclusion, the results of the experiment are entirely compatible with the notion of an impairment in the SAS of patients with PD (we make no implication as to the anatomical substrate of this hypothetical system). The theoretical concept of a limited-capacity central processor within the framework of working memory, is a useful conceptual tool for the further analysis of cognitive function in PD.

ACKNOWLEDGEMENT

The authors would like to thank Professor P. M. A. Rabbitt for allowing us access to the subject panel of the University of Manchester Age and Cognitive Performance Research Centre.

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(Received July 21, 1989 Revised February 16, 1990. Accepted March 6, 1990)

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THE MECHANISM OF SPASTIC MUSCLE HYPERTONUS

VARIATION IN REFLEX GAIN OVER THE TIME COURSE OF SPASTICITY

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SUMMARY

The electromyographic (EMG) response of the initially passive biceps brachii muscle to imposed extension applied at the elbow was studied in 19 hemiparetic and 12 normal subjects. In relaxed normal subjects, the biceps muscle was found to respond only at displacement velocities above 175 deg/s, with a single early burst of activity in the biceps EMG. In contrast, the hemiparetic subjects, in addition to the early EMG activity, also showed considerable late activity, which persisted even with stretch velocities as low as 35 deg/s. This late activity is a stretch reflex, present in fully plegic arms. It was seen in all spastic subjects in whom the tone of the biceps had been clinically assessed to be raised, but was never observed in subjects with normal muscle tone. The mean level of this EMG response was highly correlated with displacement velocity and its duration to the duration of the applied displacement. It is suggested that this reflex EMG activity is the major factor in the genesis of spastic hypertonus in the arm and that it arises not from a reduction in the threshold of the stretch reflexes of the muscle, but from a pathological increase in stretch reflex gain. It is further shown that this activity is at a high level between the first and third months after the onset of spasticity and that the reflex gain is significantly reduced when spasticity is established for a year or more. It is concluded that, while changes in passive mechanical properties may play a role when spasticity has been established for more than a year, the major cause of spastic muscle hypertonus is a pathological increase in stretch reflex activity.

INTRODUCTION

Lance (1980) has defined spasticity as '. . . a motor disorder characterised by velocity-dependent increase in tonic stretch reflexes ('muscle tone') with exaggerated tendon jerks resulting from hyperexcitability of the stretch reflex as one component of the upper motor neurone syndrome'. This definition has been widely adopted by workers in the field, but since it was made, several reports have questioned the contribution of stretch reflex activity to raised muscle tone in spastic patients. Dietz *et al.* (1981) have suggested that at the ankle joint, pathological change in the muscle fibres themselves, rather than in their activation, is the major cause of spastic hypertonus. This suggestion has been supported by reports that the amount of EMG produced in arm muscles by imposed displacement on the spastic side of hemiparetic subjects is considerably smaller than that seen on the unaffected side (Lee *et al.*, 1987). The same group has reported that

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no gain enhancement is present in voluntarily activated spastic muscle, with pathological alterations resulting from changes in the reflex threshold (Powers *et al.*, 1988) and, further, that '. . . stiffness of the initially passive spastic elbow musculature is not increased' (Powers *et al.*, 1984). These findings are, at least in part, incompatible with the definition of spasticity given above and, indeed, with many features of clinical experience. Such differences may have arisen from the fact that the above-mentioned studies were performed on voluntarily activated muscles, whereas clinical assessments of muscle hypertonus are generally made on the passive limb. The present study was an attempt to eliminate the discrepancies between the two approaches by applying rigorously controlled displacement, commonly used in neurophysiological studies of the active motor system, but to the passive limb, thus allowing direct comparison with clinical experience. Furthermore, as the mechanisms underlying spastic muscle hypertonus may not necessarily remain constant over the time course of spasticity, patients in the present study have been grouped according to the duration of their spasticity, allowing the assessment of possible changes over time. Part of these results have been reported briefly elsewhere (Fellows *et al.*, 1989).

METHODS

Experiments were performed on 19 hemiparetic subjects, all of whom had suffered a discrete unilateral lesion as the result of ischaemia in the area of the left or right middle cerebral artery (for clinical details, *see* Table); 12 normal subjects were studied as a control group.

In order to enable the assessment of possible changes in the response of the hemiparetic subjects over the time course of their spasticity, they were split into three groups on the basis of the time elapsed since their stroke occurred: group 1 (n = 5), less than 1 month; group 2 (n = 5), 1–3 months; group 3 (n = 9, more than 1 yr after stroke. All subjects gave their informed consent to the procedures involved, which were approved by the local ethical committee.

A diagrammatic representation of the displacement apparatus is shown in fig. 1A. Subjects were positioned with the arm under study laterally abducted, with the forearm and hand laying flat within a moulded plastic cast (c), and restrained by a series of straps. The upper arm was positioned so that a right angle obtained at the elbow, the subject being seated in a heavy seat, the height of which would be adjusted until the shoulder and elbow joint were at the same level. The cast restraining the forearm was, in turn, fixed to an aluminium support mounted on a bearing (b), the position of which was adjustable so that the elbow joint lay directly over the axis of rotation. When the elbow had been satisfactorily positioned, the subject's back and shoulders were supported by adjustable rests (s), thus allowing only flexion or extension movement at the elbow joint.

Displacement was monitored using a light-sensitive detector (United Detector Technology, LSC5D, Newbury Park, California), which followed the movements of a light source rigidly attached to the drive shaft. This detector yielded a signal which, after suitable processing, could resolve movements of <0.06° at the elbow, with no detectable phase shift. Displacements were applied by a powerful electric motor (Brown Boveri, MC24P, 3000 W, Heidelberg, Germany) with a four-quadrant control system (Seidel, series 5000), via a rigid coupling rod (r). This motor was strong enough to apply, for any given set of motor parameters, an identical displacement to all subjects, irrespective of arm inertia or of any resistance generated in the subject's arm. Displacement amplitude was 30°, the arm being extended from 75° to 105° of flexion. Displacement velocity was varied between 35 and 300 deg/s, the level of motor output being determined by a laboratory computer (DEC, PDP11/73). Duration of movement ranged between 175 ms and 1225 ms, depending on the selected displacement velocity. Fig. 1B shows the position and velocity curves for the 5 most commonly used speeds of displacement. The velocity curves were calculated from the position signal of the elbow by the computer. For each set of motor parameters, 10 displacements were applied at random intervals between 5 and 8 s. Subjects were instructed to remain relaxed and not to attempt to resist the displacement. This was controlled by monitoring the EMG signals (*see below*) on an oscilloscope; any trial in which a subject showed background EMG activity was discarded.

TABLE CLINICAL DETAILS OF THE HEMIPARETIC SUBJECTS

Case	*Sex*	*Age (yrs)*	*Localization of ischaemia*	*Paresis*[1]	*Tone*[2]	*Mean biceps EMG (μV)*[3]
Group 1 (onset <1 month)						
1	M	65	R MCA	2	0	0
2	M	66	R MCA	0	0	1.8
3	M	59	R MCA	0	0	0
4	F	39	L MCA	4	1	3.2
5	M	41	L MCA	0	1	14.7
Group 2 (onset 1–3 months)						
6	F	19	L MCA	2	1	7.5
7	F	49	R MCA	0	3	31.3
8	M	65	R MCA	0	2	22.6
9	M	76	R MCA	0	4	58.6
10	M	57	L MCA	2	3	18.6
Group 3 (onset >1 yr)						
11	M	33	R MCA	3	2	12.1
12	F	48	L MCA	0	4	6.9
13	M	47	L MCA	4	4	19.1
14	F	50	L MCA	0	4	11.6
15	F	50	L MCA	3	4	5.7
16	F	41	R MCA	0	1	4.0
17	F	61	R MCA	0	1	6.3
18	F	24	R MCA	2	4	34.5
19	M	36	R MCA	3	3	12.1

L MCA = left middle cerebral artery, R MCA = right middle cerebral artery
[1] Paresis of the affected arm, expressed according to the MRC scale: 0 = no voluntary power, 1 = visible contraction without affect; 2 = movement without the influence of gravity, 3 = movement against gravity; 4 = movement against resistance; 5 = normal power. [2] Tone of the spastic arm, expressed on the Ashworth scale: 0 = normal; 1 = slight increase in tone, giving a 'catch' when the limb is moved, 2 = more marked increase in tone, but limb easily flexed; 3 = considerable increase in tone—passive movement difficult, 4 = limb rigid in flexion or extension. [3] Mean biceps EMG level at 240 deg/s. For details of analysis, *see* text

Electromyograms were recorded from over the biceps brachii and triceps brachii muscles of the perturbed arm using custom-built surface electrodes which contained a small preamplifier and were designed to reduce movement artefacts to a minimum. The signals so obtained were amplified and filtered (bandwidth 20 Hz – 1 kHz), then passed, along with the position signal, to the ADC boards of the computer, which sampled at a rate of 1 kHz per channel. Both EMGs were then rectified and an average constructed from the 10 displacements applied at each set of motor parameters. Position, raw EMG data and the motor control pulses were also recorded on tape (Racal, Store 7D, Elektro-Automatik, Düsseldorf).

At the start of each experimental series, tendon jerk reflexes were elicited from the biceps and triceps muscles, using a reflex hammer containing a small microswitch which activated computer sampling. Five responses were obtained from each muscle, which were then passed to the computer, which rectified the signal and constructed an average. The integrated EMG (μV.s), onset latency and duration were then determined.

For the displacement experiments, the averaged responses obtained for each displacement velocity were analysed off-line to yield the integrated EMG value, onset latency and duration of the responses. It should be noted that latencies were determined from the first deflection in the velocity record, not from the control pulse to the motor, which preceded the actual onset of movement by 12 ms.

The significance of differences between the groups was assessed using an analysis of variance of regression coefficients over groups. The analysis was performed using program IR of the BMDP statistics package (BMDP Statistical Software, Los Angeles).

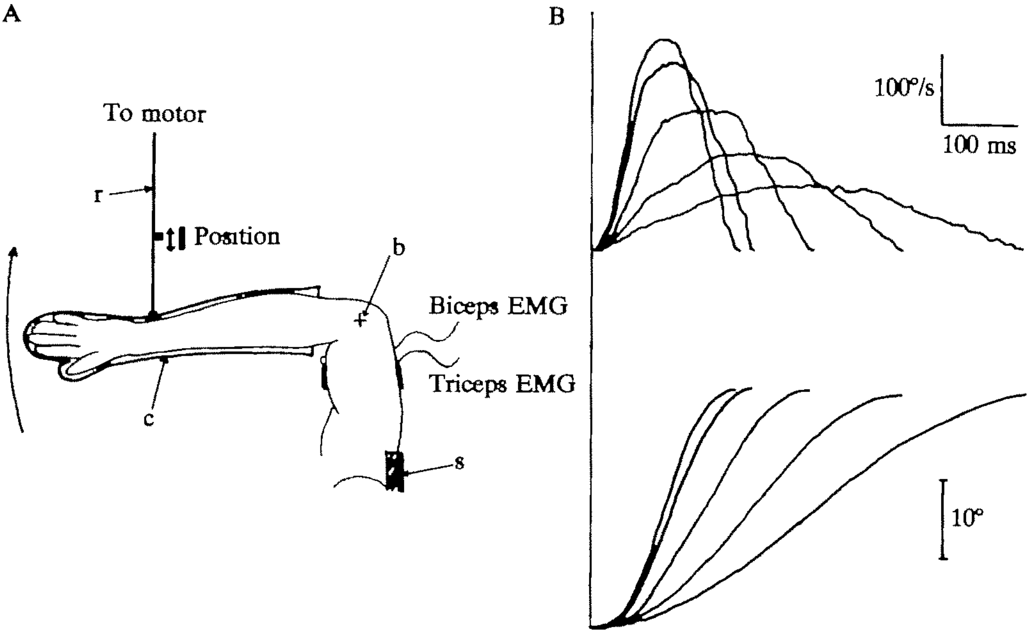

Fig 1. A, diagrammatic representation of the apparatus. For explanation of symbols, *see* Methods B, position (*lower*) and velocity (*upper*) traces for the 5 most commonly applied displacement velocities.

RESULTS

Extension of the elbow by 30° with a displacement velocity greater than 200 deg/s was found to elicit a reflex response in the relaxed biceps of 6 out of 12 of the normal subjects. In the remainder, no response was elicited, even at the fastest stretch velocities applied (300 deg/s). Fig. 2A shows the responses of the normal subject with the most active reflex. Each trace is the rectified and averaged biceps EMG response obtained from 10 displacements at the velocity given beside the trace. The length of each trace indicates the duration of the displacement. The corresponding joint position traces are shown in the centre of the figure. This subject continued to respond until the stretch velocity fell below 175 deg/s, as opposed to the threshold of 200 deg/s observed in the other normal subjects who showed a response. The timing of the responses of this subject, however, is representative of the normal group. It may be seen that the response consisted of a single synchronized burst of activity (average latency at 300 deg/s: 61 ms, SE ±4, duration 46 ms, SE ±3). The average area of the rectified EMG response (0.68 μV.s, SE ±0.18) was about 50% of the average biceps tendon reflex (1.36 μV.s, SE ±0.48, average latency 10.8 ms, SE ±0.55) although, surprisingly, the size of an individual's response to imposed displacement was not systematically related to the size of the subject's tendon reflex. As displacement velocity is reduced, it is apparent that the biceps EMG response decreases in amplitude and appears at a progressively longer latency. The end of this burst, however, remained relatively constant, with the net result that duration of the burst was reduced as displacement velocity fell. None of the normal subjects developed, in the absence of voluntary activation of the biceps, any EMG activity

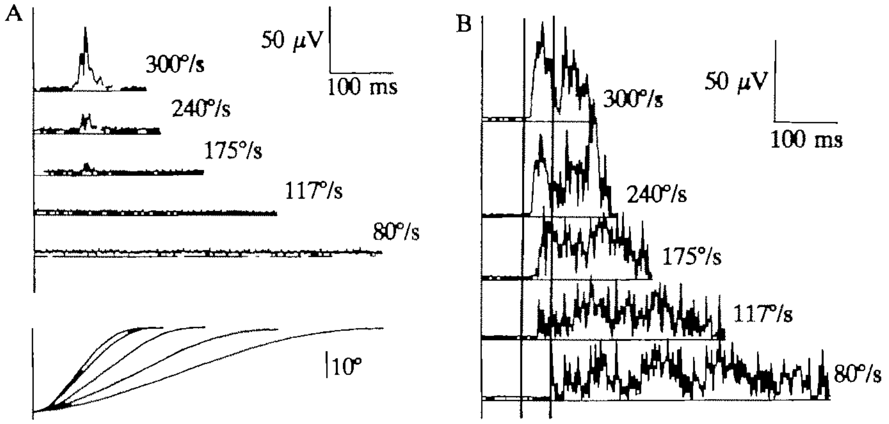

FIG 2. The response of the biceps muscle of a normal (A) and a spastic (B) human subject (group 3, >1 yr) to a 30° extending displacement applied at the initially relaxed elbow. Each trace represents the rectified and averaged EMG record obtained from 10 displacements at the velocity specified beside it. The length of a trace corresponds to the duration of the displacement, the position records for which are shown between the two sets of EMG records The dotted lines in B indicate the timing of the 'normal range' (*see* text for definition)

before or after this synchronized burst. In subsequent descriptions, the average onset latency and duration of the response of the normal subjects to a 300 deg/s displacement will be referred to as the 'normal range' (61 – 107 ms) and activity seen in the hemiparetic subjects occurring outside these limits will be taken to be abnormal. Clearly, for lower velocities of displacement, when the onset latency tended to move later, use of this range may result in some misclassification of earlier activity as normal. Activity occurring later than the normal range, however, can be defined with confidence, as the end of the normal activity remained relatively constant, independent of displacement velocity.

Fig. 2B shows a typical set of biceps EMG responses for a hemiparetic subject from group 3 (>1 yr) elicited by elbow extension on the spastic side at a range of displacement velocities. The dotted lines define the 'normal range' (*see above*) and, as for the normal traces shown in fig. 2A, the length of the EMG trace reflects the duration of the displacement. It is evident that the pattern of response in the biceps EMG is far more complex than that of the normal. While a considerable component of the biceps EMG occurs within the 'normal range', later activity is clearly apparent. This particular subject did not show any activity earlier than the normal range, but such activity was often seen in both the biceps of the affected side and the triceps of the 'unaffected side' of hemiparetic subjects. This early activity has, however, been dealt with more fully in an earlier paper (Thilmann *et al.*, 1990) and this report will concern only the later components.

While at the fastest rate of displacement (300 deg/s) the end of the 'normal range' and the start of the later activity was not always clearly defined, at lower displacement velocities a clear demarcation was seen in the EMG record: at the higher velocities of stretch, the EMG showed a reduction in activity at a timing around the end of the 'normal' range, while at the lower velocities which evoked no early activity, the start of the late activity was clearly visible at or shortly after the end of the 'normal' range. This late activity was not synchronized into bursts, but showed a rather tonic pattern which terminated at around the time that the applied elbow extension was completed, usually slightly before, due to the velocity profiles of the applied stretch (*see* fig. 1B), which were low in the last fraction of the movement, as the computer braked the system. Tonic biceps EMG activity was never observed in the subsequent hold phase of the displacement. This late activity continued to be evoked even at displacement velocities at which the earlier components might no longer be evoked. This is apparent in fig. 2B, in which the earlier components clearly disappear when the displacement velocity falls below 117 deg/s, whereas the later activity is still visible with the lowest displacement velocity illustrated (80 deg/s), and was often still visible at velocities as low as 35 deg/s, which was the lowest displacement velocity applied. This abnormal late activity was seen in all hemiparetic subjects who had been assessed by a clinician to have raised tone in the biceps. Other hemiparetic subjects, not included in the present data, who had been assessed to show no increase in tone in the biceps showed a response similar to that obtained in the normal subjects, consisting of a single burst in the biceps EMG, which disappeared if displacement velocity was lower than 175 deg/s.

In order to quantify this later activity, EMG values were expressed as a mean level (in μV). For this purpose, the integral of the total rectified biceps EMG occurring later than the 'normal range' was computed. Where necessary, a correction was made for background electrode noise by subtracting the noise level present before the onset of displacement. The resulting value was then divided by the duration of the late activity. Fig. 3A shows the mean biceps EMG level for this activity obtained from group 3, plotted against displacement velocity. It may be seen that the mean level of biceps EMG is directly proportional to the displacement velocity, the relationship showing a significant linear correlation ($r = 0.998$, $P < 0.0012$).

As mentioned above, a close correspondence also existed between the end of the late biceps EMG activity and the end of the displacement. This relationship, which was highly linear, is illustrated in fig. 3B. This shows the average value for the end of the late EMG activity for the 9 subjects of group 3, plotted against the duration of the elbow extension. The linear regression line is shown; this correlation was highly significant ($r = 0.9998$, $P < 0.001$).

Although the level of biceps EMG generated is closely linked to displacement velocity and its duration to the duration of extension, the onset of the activity is less clearly linked to the displacement parameters. In a small minority of subjects, the onset of the late activity was undoubtedly later as displacement velocity was reduced. Such a response is shown in fig. 4, and it is clear that the latency of the activity increases progressively as the displacement becomes slower, with the biceps first beginning to respond, at a displacement velocity of 40 deg/s, more than 400 ms after the onset of extension. In this subject, the onset of the activity appears to be linked to a position threshold. In the majority of subjects, however, the pattern was that shown in fig. 2B, with the onset latency of the late activity remaining constant (for group 3, 107 ms, SE ±3), irrespective of displacement velocity and independent of the position of the arm.

In the two groups of hemiparetic subjects (group 1, <1 month and group 2, 1–3 months) studied earlier in the time course of spasticity, later activity was also apparent in the biceps EMG of the affected side, but this activity showed some differences from that of subjects in whom spasticity was well established (group 3). Evidence of such activity was only rarely apparent in the first month after the stroke. In some cases this

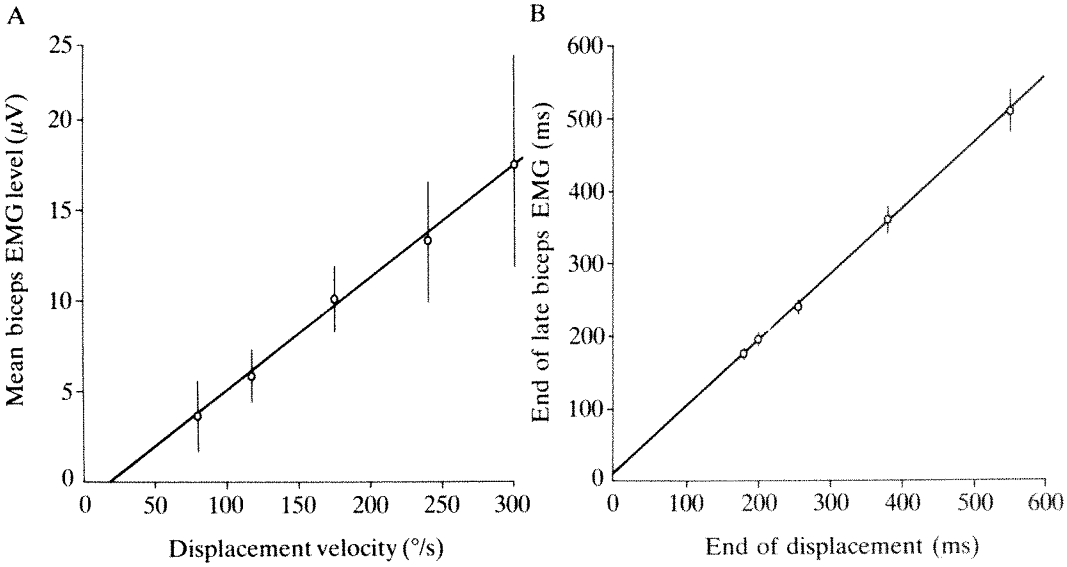

FIG. 3. Linear correlations between displacement velocity and the mean biceps brachii EMG level of the late response (A) and between the time at which the late activity terminated and the time that displacement ceased (B). Each point is the average value (±SE) obtained from the 9 subjects of group 3, in whom spasticity had been established for 1 yr or more.

preceded the reappearance of short latency reflexes, but more generally the later activity developed concurrently, becoming apparent as a continuation of the 'normal range' without a clearly defined separation into early and late components. Its duration increased as the displacement velocity was reduced, but it did not last for the entire duration of the displacement (average duration at 300 deg/s, 49±11 ms for group 1; 67±5 ms for group 3). Furthermore, the threshold for the appearance of this late activity was higher, with it being seen only at displacement velocities above 150 deg/s. The mean EMG level was low and showed no correlation with the velocity of stretch.

By the start of the second month after the onset of spasticity (group 2), however, this late activity was in almost all cases fully established, with its timing and threshold indistinguishable from that of the response, fully described above, of the subjects in group 3.

Although the subjects of group 2 showed a response pattern qualitatively similar to that of group 3, in quantitative terms they showed significantly greater mean biceps EMG levels than those subjects in group 3. The mean level of the late EMG activity was significantly correlated to displacement velocity in both groups (group 2, $P < 0.0011$; group 3, $P < 0.0012$). As the plot of response magnitude against displacement velocity represents the input/output relationship for this response, the respective gradients of the regression lines yield the response per unit input, that is, the reflex gain in the relaxed arm of spastic subjects. Thus the difference between the regression lines fitted to the two groups, which is statistically significant ($P = 0.0067$), indicates that the gain of the motor system in hemiparetic subjects alters during the time course of spasticity, reaching a high level in the second and third month after the onset of spasticity, then reducing as spasticity becomes more established. Furthermore, although normal subjects do not display this late activity in the relaxed state, and their gain at rest cannot therefore be directly determined, it is apparent that as the spastic subjects show different gains at different times during the time course of spasticity,

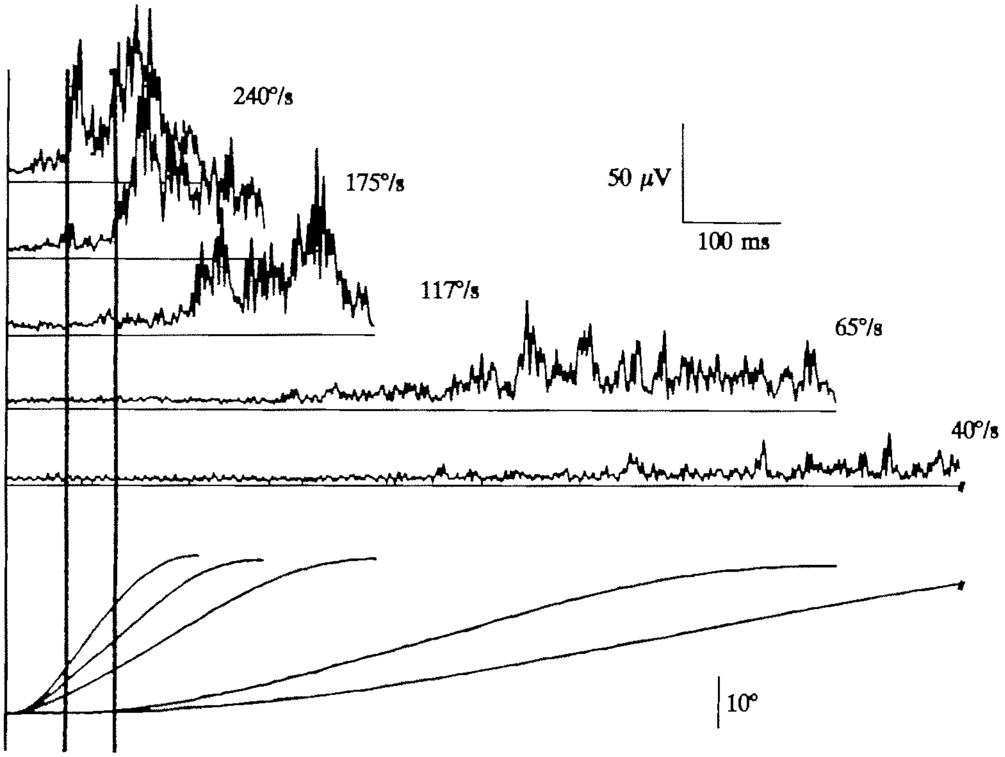

FIG. 4. The responses of the spastic biceps brachii of a patient from group 3 (>1 yr) to extending displacement at a range of velocities Each trace is the mean of the rectified EMG obtained from 10 displacements. The length of each trace represents the duration of displacement (except for the 40 deg/s trace, which has been terminated in order to allow the same time scale to be used for all traces), with the corresponding position traces being shown below. The dotted lines indicate the limits of the 'normal range'.

on one or both of these occasions the gain of relaxed motor system on the affected side of hemiparetic subjects must be significantly different from the gain at rest of normal subjects.

DISCUSSION

The present study has demonstrated that, in hemiparetic patients with clinical signs of raised muscle tone, imposed extension of the relaxed elbow on the affected side produces, in addition to early activity, a considerable late and prolonged activation of the biceps muscle, never seen in normal subjects, which lasts for as long as displacement is applied. The level of this EMG activity increases as stretch becomes more rapid, showing a positive linear correlation with the velocity of the displacement. Thus this late activity occurs in association with and shares the properties of clinically described muscle hypertonia. It is therefore tempting to attribute the genesis of the raised tone of spastic muscle to this EMG activity. Before doing so, however, the nature of this response must be considered.

The onset of latency of this response (107 ms, SE ± 3) falls into the range (130 ms ± 22) of the voluntary reaction time for the biceps in normal subjects (Cordo and Nashner, 1982), but the late activity is unlikely, for the following reasons, to result from voluntary intervention by the patients and may be taken to be a stretch reflex: the late activity was present in subjects in whom the arm muscles were fully plegic and voluntary activation, therefore, impossible. Even in paretic patients, in whom some degree of voluntary intervention is theoretically possible, the voluntary contribution must be negligible, as qualitatively identical patterns of late activity were obtained in plegic and paretic patients and, furthermore, those subjects in whom spasticity had been established for more than a year showed less EMG activation than subjects in whom spasticity was between 1 and 3 months old, despite many of the former group having recovered considerably more voluntary control of the arm musculature. It is thus justifiable to consider this late activity to be reflex in nature. The latency of this response was fixed in the overwhelming majority of subjects and, given the late onset, it would seem likely that it must be mediated over a predominantly polysynaptic pathway. Which afferents are responsible for its generation is not immediately clear, but the demonstrated velocity sensitivity and the fact that its duration is significantly correlated to that of the applied displacement suggests strongly that its continuation is dependent on ongoing proprioceptive input, presumably from muscle spindle afferents.

The properties of this late activity are well matched with clinical definitions of spastic hypertonus and with the results of earlier studies which measured EMG responses to manually applied displacement (Rushworth, 1960; Ashby and Burke, 1971). Such late reflex activity in response to large amplitude displacement has, in the past (e.g., Neilson, 1972), been referred to as a tonic stretch reflex (TSR). Some confusion exists, however, about the definition of such activity, namely as to whether it is a response to 'maintained' (i.e., static) or 'steady' (i.e., constant velocity) stretch. Given this confusion and the fact that the reflex activity observed in the present study was never seen after displacement had ceased, it is perhaps wiser to avoid the use of the term TSR in the present case. It is, however, clearly somewhat cumbersome to continue to refer to it as 'late' reflex activity, and a compact term of reference would be an advantage. As this activity is seen, in the initially passive state, exclusively in spastic subjects, we would suggest that it be referred to as the 'spastic stretch reflex' (SSR).

It is clear that the SSR of the spastic biceps is the major component of spastic muscle hypertonus. It was invariably seen in those subjects who had been clinically assessed to have raised muscle tone and in these subjects alone. It would not, however, be justified to suggest that alteration in the mechanical properties of muscle plays no role in spastic hypertonus: from the clinical details of the patients (*see* Table), it is apparent that, despite the reduction in the level of the EMG responses in those patients with established spasticity, muscle tone, assessed on the Ashworth scale (Ashworth, 1964) was, if anything, higher in the subjects of group 3 than those of group 2. Given the highly subjective nature and somewhat coarse scaling of this index, however, the significance of this discrepancy is open to question. Nevertheless, it may be that, as spasticity becomes established, another factor begins to play a role in hypertonia. It has been suggested that, in the case of hypertonia in the leg muscles, changes occur in the mechanical properties of spastic muscle (Dietz and Berger, 1983), a suggestion not contradictory to the present findings. To suggest, however, as this group has done, that changes in passive muscle properties are the major cause of spastic muscle hypertonus, with reflex hyperexcitability playing no role, is not justifiable in the upper limb, in light of the present findings. Whether changes in the passive mechanical properties of the limb, which became more apparent when spasticity has been established for a year or more, result from pathological mechanisms, or simply from stiffness caused by disuse, remains to be discovered.

It is also a possibility that the apparent discrepancy seen in group 3 between clinically assessed tone and the level of EMG activity recorded during limb displacement may arise from physiological changes in the motor units of the spastic biceps, resulting in a change in the EMG/force ratio. Young and Mayer (1982) have reported that motor units in spastic subjects show both prolonged twitch times and increased twitch tensions. This being the case, it might be expected that, although the gain of the reflex system had reduced in the patients of group 3, leading to lower levels of EMG activity, a given level of EMG would produce more force, resulting in the high levels of muscle tone seen clinically in these patients.

Recent reports have suggested that the primary cause of spasticity is a lowering of the threshold of the stretch reflex, without significant enhancement of gain (Lee *et al.*, 1987; Powers *et al.*, 1988). There are, however, several reasons for believing that these conclusions were drawn from studies which did not directly address the problem of spastic muscle hypertonus, as it is classically defined. First, these studies were performed on voluntarily active muscle, under which conditions normal muscle also shows enhanced EMG activity over the duration of the displacement. Neilson (1972) has shown that the gain of late reflex activity in the voluntarily activated spastic biceps remains fixed at a relatively high level, irrespective of the level of voluntary contraction. In the normal biceps, however, the gain of this activity increases steeply with increasing voluntary activation, reaching similar values to that of spastic muscle at moderate levels of contraction. Secondly, Rack *et al.* (1984) have found that, for the voluntarily activated triceps surae muscle, the magnitude of the reflexes of spastic subjects, while large, was within the high end of the normal range. Thus a definition of 'enhanced' gain could depend very much from where in this extensive normal range the control group lay: a control group composed largely of normal subjects with relatively inactive stretch reflexes would obviously make changes in the spastic group more apparent than a control group whose reflexes were brisk. Both these findings would lead one to predict that the experimental paradigm used by Lee *et al.* (1987) would fail to uncover significant differences in the gain of the late reflex activity in the biceps between normal and spastic subjects.

It has further been demonstrated (Neilson and Andrews, 1973), that the properties of late stretch reflex activity in the biceps muscle of athetotic patients are markedly different in the active and passive states. Thus it is erroneous to apply a hypothesis developed for activated musculature to the passive state. It is precisely in this latter state, however, that spastic and normal subjects show some of the most dramatic differences: the former, as the present findings emphasize, develop late reflex EMG activity in initially passive muscle, a situation never observed in the normal subjects. It is clearly difficult to compare the gain of this reflex activity between normals and spastics under relaxed conditions, i.e., those under which spastic muscle hypertonus is usually defined, because a qualitatively new reflex is present in the spastic subjects. To attribute the major role in the genesis of this difference between the normal and the spastic to threshold changes (Powers *et al.*, 1989), however, leads to some unlikely conclusions: if the whole range of the responses in the spastics is merely the 'normal range', shifted in threshold, then the normal response must occur at ridiculously high displacement velocities, given that no response was seen in normals in the present study with displacement velocities as high as 300 deg/s, or, in response to manually applied stretches at rates as high as 500 deg/s (Ashby and Burke, 1971). It is thus clear that changes in threshold alone cannot explain the genesis of the late reflex biceps EMG activity in spastic subjects.

That some change in the threshold of the stretch reflexes does occur is, however, apparent. The shorter latency reflex components in the spastic biceps continue to appear at lower displacement velocities (over 100 deg/s for spastics, but over 175 deg/s for normals) than in the normal subjects (Thilmann *et al.*, 1990). Furthermore, during the first month of spasticity, the SSR appears at first only with rapid displacement, but soon becomes apparent at much slower displacement velocities. After the first month of spasticity, however, this threshold remains relatively fixed. Further changes occur only in the magnitude of the response, which are maximal in subjects whose spasticity had been established between 1 and 3 months (group 2), then show a significant reduction a year after the onset of spasticity (group 3). Although, as stated, the absence of an SSR in normals makes direct comparison of the gain of the reflex system difficult, the demonstrated difference in the gain of the response during the time course of spasticity allows this problem to be circumvented: whatever the gain of the normal system, it cannot be equal both to that of the spastic subjects of group 2 *and* to that of those of group 3. It follows, therefore, that a change in the gain of the stretch reflex of spastic subjects has occurred. This reconfirms the classical view that spastic muscle hypertonus results primarily from enhanced stretch reflexes.

ACKNOWLEDGEMENTS

We wish to thank Professor C. G. Phillips for the gift of parts of the mechanical equipment, U. Arenz for technical assistance and H. F. Ross for computer software and for statistical advice. Professor J. Noth made helpful comments on the manuscript. This work was supported by the Deutsche Forschungsgemeinschaft. S.J.F. was in receipt of a Research fellowship from the Alexander von Humboldt Stiftung.

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(Received September 19, 1989. Revised February 7, 1990 Accepted March 6, 1990)

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REVERSED LATERALIZATION OF COGNITIVE FUNCTIONS IN RIGHT HANDERS

EXCEPTIONS TO CLASSICAL APHASIOLOGY

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SUMMARY

Most current and past research on the cerebral organization of cognitive functions has presupposed certain specialized hemisphere operations. At least for right handers, language and praxis are to be organized in the left hemisphere, while affective prosody, configurational spatial capacity, and global attention are lateralized in the right hemisphere. Deviations from these presuppositions, as in crossed aphasics and perhaps left handers, are generally considered to be 'exceptions' and either to disprove the rules or to be irrelevant to the rule. We report 4 very 'exceptional' cases, right handers with almost entirely reversed lateralization of functions. Analysis of the *intra*hemispheric relationships between functions suggests that there may be a specific neurobiology to the interrelationships between and among cognitive functions, handedness, and the intrahemisphere localization of the function.

INTRODUCTION

Theories regarding the relationship between handedness and the lateralization of brain functions have been approached from several frames of reference. Differences between right and left handers in the profile of cognitive impairments after brain injury have been the most accessible avenue available thus far for analysis of the relationships between handedness and lateralized cognitive operations (Hécaen and Sauguet, 1971; Borod *et al.*, 1985). The most commonly utilized dimension of this analysis thus far has been to study the differences, if any, between the aphasia profiles of right and left handers after lesions of either hemisphere (Goodglass and Quadfasel, 1954; Gloning *et al.*, 1969; Hécaen and Sauguet, 1971; Basso *et al.*, 1985*a*; Naeser and Borod, 1986). Aphasia has probably been selected as the arena for analysing the relationship of handedness to the lateralization of cognitive functions for two reasons. First, there is the robust relationship of the left hemisphere to right handedness and of left hemisphere lesions to aphasia; exceptions to these dual relationships might be very informative about any mandatory relationships of hemisphere, handedness, praxis and language. Secondly, there are several proven methodologies for establishing relatively clear dissociations in a number of language functions.

It is possible that similar dissociations occur in functions served by the right hemisphere, but this has been less vigorously investigated (Hécaen and Ajuriaguerra, 1963; Kriendler

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et al., 1966; Hécaen and Sauguet, 1971). There is ample evidence that the right hemisphere has specialized roles in processing higher order emotional information (Gainotti, 1972; Ross, 1981), integrating the overall perceptual configuration of visuospatial material (Paterson and Zangwill, 1944; Benton, 1967; Newcombe and Russell, 1969; Mehta *et al.*, 1987; Delis *et al.*, 1988), and maintaining and sustaining appropriate attention and concentration (Mesulam, 1981). These observations suggest that there may be discrete lateralized nonverbal functions that might serve as appropriate dependent measures in analyses designed to determine relationships between hemisphere, handedness and these additional cognitive and behavioural functions.

There is considerable importance for the development of deeper understanding of these relationships beyond the simple curiosity value of reporting anomalous cases. One important practical value is the actual explanation of anomalous cases. It is difficult to sustain comprehensive theories about the cerebral organization of language or cognitive functions in the face of anomalous cases, so-called 'exceptions' (Basso *et al.*, 1985*b*), without a theory (or theories) that accounts for the anomalies. A second important value is the potential description of how different cognitive and behavioural operations are related to each other. For this, the study of anomalous cases may be the only practical approach. When one or another cognitive function is unexpectedly displaced from its usual hemisphere of operation, what other functions follow it? Do their intrinsic characteristics alter when they are dislocated to the unexpected hemisphere?

We have previously reported 2 cases of crossed aphasia and an extensive review of this topic in an attempt to begin to define the possible relationships between the lateralization of language and the lateralization of other cognitive operations (Alexander *et al.*, 1989). We now report 4 right-handed patients with lesions of the left hemisphere. They demonstrated dissociations between neuropsychological functions usually held to be related and lateralized to the left hemisphere. They also had completely reversed lateralization of functions usually held to be associated with the right hemisphere. It is our hypothesis that by extending the dimensions of assessment to include all lateralized functions, that is, oral and written language, buccofacial and limb praxis, visuospatial skills, attention, affect (including prosody), and emotional functioning, it may be possible to clarify examples of anomalous lateralization. By accounting for 'exceptions' this could lead to improved stability of theories of basic brain-behaviour relationships. There is also the possibility of sharpening our understanding of the basic neurobiology of normal lateralization.

CASE REPORTS

Case 1

This 62-yr-old woman had abrupt onset of right-sided weakness. The initial evaluation included a normal ECG and cranial CT scan. Her past medical history was unremarkable except for episodic palpitations. While in hospital paroxysmal atrial fibrillation was documented. She had minimal neurological improvement before transfer for rehabilitation. We examined her at 2 wks and 8 wks after onset. She considered herself to be primarily right handed. Six of 8 unimanual tasks were performed exclusively with the right hand. She brushed her hair and her teeth with her left hand, allegedly due to toiletry instruction from a left-handed governess. No other unimanual tasks were ever performed with the left hand. Her parents and her 2 children were exclusively right handed; she had no siblings. There was no personal or family history of early life brain injury, seizures or learning disability. She was a college graduate.

Neurological examination demonstrated a severe spastic right hemiparesis maximal in the lower face and entire upper extremity. There was severe right-sided sensory loss. No visual field defect was demonstrable on confrontation, but there was right-sided inattention on simultaneous bilateral stimulation. There was moderate hypophonia, but no dysarthria. The patient's capacity to produce emotional colouring in her voice ('affective prosody'; Ross, 1981) was measured independently by 3 of the authors. Each judged her affect prosody to be severely reduced. There was no buccofacial apraxia, but there was spatial/body part as object errors (Goodglass and Kaplan, 1963) on 3/10 of limb tasks with the left arm. She was unable to produce any melodic line while attempting to sing familiar tunes. At 8 wks postonset, neurological examination was unchanged except that her right-sided inattention to simultaneous bilateral stimulation had cleared.

A CT scan performed 5 wks after onset demonstrated a large infarct in the left hemisphere (fig. 1). There was substantial involvement of the frontal operculum, middle frontal gyrus, insula, anterior temporal lobe, motor and sensory cortices, inferior parietal lobule, putamen, and the white matter deep to the involved cerebral regions down to and including the frontal isthmus and the entire anterior and middle periventricular white matter (Alexander *et al.*, 1987). There were no lesions in the right hemisphere. Cerebral asymmetries were typical (Koff *et al.*, 1986), that is, the left posterior brain was longer and wider than the right. This is the most common cerebral asymmetry.

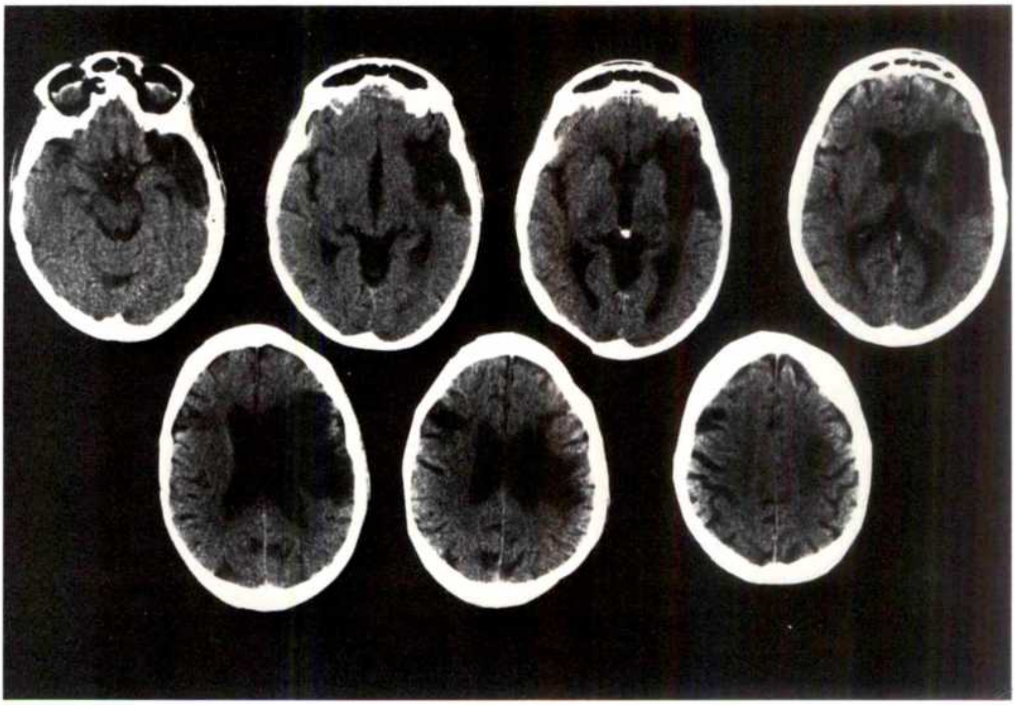

FIG. 1. CT scan performed 5 wks postonset in *Case 1*. There is a large infarct involving much of the territory of the left middle cerebral artery, extensively damaging the frontal operculum, lower motor cortex, posterior middle frontal gyrus, inferior parietal lobe, anterior temporal lobe, insula, lateral putamen and the entire periventicular white matter.

The Western Aphasia Battery (WAB) was administered 2 wks postonset and demonstrated no language abnormalities (Table 1). Spontaneous verbal output was fluent and grammatical with appropriate informational content. There was no evidence of paraphasic errors and/or word finding difficulties. Auditory

TABLE 1 LANGUAGE EXAMINATIONS

	Case 1	*Case 2*		*Case 3*		*Case*	
	WAB	*BDAE*		*BDAE*		*BDAE*	
		1 1 88	*16 12 88*	*1 4 88*	*9 5 88*	*15.11 88*	*30 1 89*
Spontaneous speech							
Information content	10/10	Appropriate		Confabulatory, tangential		Appropriate	
Fluency, grammatical comp , paraphasias	10/10	Normal		Normal		Normal	
Oral expression							
Automized sequences	Good	8/8	8/8	8/8	8/8	6/8	7/8
Recitation, singing, rhythm	↓ Prosody	3/6	4/6	↓ Prosody	↓ Prosody	↓ Prosody	↓ Prosody
Auditory comprehension							
Yes/no questions	60/60	–	–	–	–	–	–
Auditory word recognition	60/60	–	–	–	–	–	–
Word discrimination	–	18/72	36/72	7/24	24/36	–	–
Body part identification	–	20/20	20/20	0/3	0/3	17/20	18/20
Commands	–	11/15	15/15	0/3	4/5	9/15	12/15
Complex ideational material	–	9/12	12/12	NT	1/12	6/12	12/12
Repetition							
Repetition of words	100/100	10/10	10/10	8/10	9/10	10/10	10/10
Repetition of phrases							
High probability	–	8/8	8/8	8/8	7/8	7/8	NT
Low probability	–	3/5	3/5	6/8	4/8	8/8	NT
Naming							
Responsive naming	10/10	24/30	30/30	11/30	15/30	28/30	30/30
Visual confrontation naming		90/114	114/114	44/114	81/108	42/42	NT
Boston Naming Test	59/60	21/60	40/60		17/60		48/60
Reading							
Words	–	30/30	30/30	30/30	30/30	NT	8/10
Sentences	40/40	NT	10/10	9/10	9/10	NT	8/10
Commands	20/20	–	–	–	–	–	–
Reading comprehension							
Symbol and word discrimination	Normal to informal testing	10/10	10/10	0/4	4/10	–	–
Word recognition		8/8	8/8	2/8	1/8	–	–
Comprehension of oral spelling		0/8	NT	6/8	6/8	5/8	6/8
Word to picture matching	–	5/10	8/10	0/4	1/8	–	–
Reading sentences and paragraphs	–	2/5	3/5	2/5	2/5	NT	5/5
Informational							
Word to picture matching	–	–	–	1/5	4/5	–	–
Word to recognition	–	–	–	5/5	5/5	–	–
Writing							
Dictation	10/10	0	0	0	0	4/10	5/10

NT = not tested

comprehension, repetition, and naming were intact. Writing (with the left hand) was compromised by reduced legibility, but spelling and grammatical structure were adequate. Reading was normal.

Neuropsychological testing was performed 6 wks after onset and demonstrated deficits in attention, spatial function, insight and higher level functioning (Tables 2 and 3). On the WAIS-R, the patient achieved a VIQ of 109, a PIQ of 87, and a FSIQ of 98. The 22 point discrepancy between her verbal and performance scores reflects relatively poor visuospatial skills. She had difficulty finding missing parts of pictures (Picture Completion subscale score (SS) = 5), appreciating part-whole relationships as demonstrated by the block design (SS = 7) and object assembly (SS = 7) subtests, as well as planning and sequencing pictures into meaningful stories (Picture Arrangement SS = 5). On the block design subtest, she broke configuration only on design 7. On the object assembly subtest, she had most difficulty with those puzzles whose elements were not perceptually obvious (hand and elephant)

TABLE 2 NEUROPSYCHOLOGICAL TEST FINDINGS

	Case 1	*Case 2*	*Case 3*	*Case 4*
Praxis				
Limb	Mild	Severe	Severe	Normal
Buccofacial	Normal	Severe	Severe	Normal
Attention				
Digit span	6 F 5 B	7 F 4 B	6 F 2 B	6 F 2 B
Corsi Block	6 F 4 B	4 F 3 B	3 F 2 B	2 F 2 B
Mental control (WMS)	5/9	6/9	3/9	2/9
Auditory As	25/26 1 FP	25/26 1 FP	20/26 7 FB	22/26
Auditory XIs	19/26 1 FP	24/26	5/26	16/26
Motor impersistence	2/7	4/7	0/7	0/7
Visuospatial				
Benton Visual Form Discrimination	10/16, R neglect	12/16, R neglect	0/16, R neglect	5/16, R neglect
Judgement of Line	25/30	8/30	0/30	0/30
Map	14/14	12/14	0/14	1/14
Line bisection	19/19	19/19	3/19	7/19
Construction	Disorganized, overdrawn	Segmented, R neglect	Segmented, loss of configuration	Segmented
Insight	None	None	None	None
Behaviour	No concern	No concern	No concern	No concern
Affect	Flat	Flat	Flat	Flat

Visuoperceptual skills were mildly impaired. She had difficulty matching geometric designs (Benton Visual Form Discrimination Test 10/16), but her performance on the Benton Judgement of Line Test was well within normal limits for age (26/30). There was no evidence of neglect on line bisection or visual cancellation tests, although she was slow to scan to the right. She could identify 14/14 map locations. Drawings from copy of simple and complex geometric designs were disorganized, simplified and quite overdrawn.

Performance on tests of attention was poor. Although she scored in the 'average' range on the digit span subtest of the WAIS-R (SS = 10), she was unable to maintain her attention on an auditory vigilance test (Auditory XIs 19/26) nor sustain simple motor movements on a motor impersistence task (2/7). Mental control was also poor in that she was unable to perform serial 3 addition without loss of set. On Wisconsin Card Sorting, she was unable to achieve any categories in 128 cards and 74% of her errors were perseverative. She showed an apathetic and unconcerned attitude throughout her hospitalization and had no appreciation of her deficits or potential limitations. Affect was extremely flat; she showed no spontaneous emotion. This emotional indifference was reflected on the Zung Self-Rating Depression Scale when she denied depression or any feelings of hopelessness.

Case 2

This 57-yr-old man developed right-sided weakness and confusion. The acute events are not known; he was found by family. Initial medical evaluation included a normal ECG and CT cranial scan; carotid artery Doppler sonography documented left internal carotid occlusion. There was little change in his condition before transfer for rehabilitation. We examined him at 2 and 8 wks postonset.

He was exclusively right handed for all unimanual tasks (writing, toiletry, tool usage, and recreational pursuits). His parents and 2 siblings were right handed; he had no children. There was no personal or family history of early life brain injury, seizures or learning disability. He had a long history of alcohol abuse with several minor head injuries; none required hospitalization or was ever known to produce any neurological sequelae. He was a high school graduate.

Neurological examination at 2 and 8 wks after onset demonstrated a severe flaccid right hemiplegia, severe right-sided sensory loss and a probably complete right hemianopia. There was marked buccofacial and limb apraxia. There was no dysarthria or hypophonia. Severe affective dysprosody (determined in the same manner as Case 1) was present. He was unable to produce any melody of familiar songs.

A CT scan performed 6 wks after onset demonstrated a large left hemisphere infarct (fig. 2). Damage was very similar to that in Case 1. There was no right hemisphere lesion. Cerebral asymmetries (as for Case 1) were typical (Koff *et al.*, 1986).

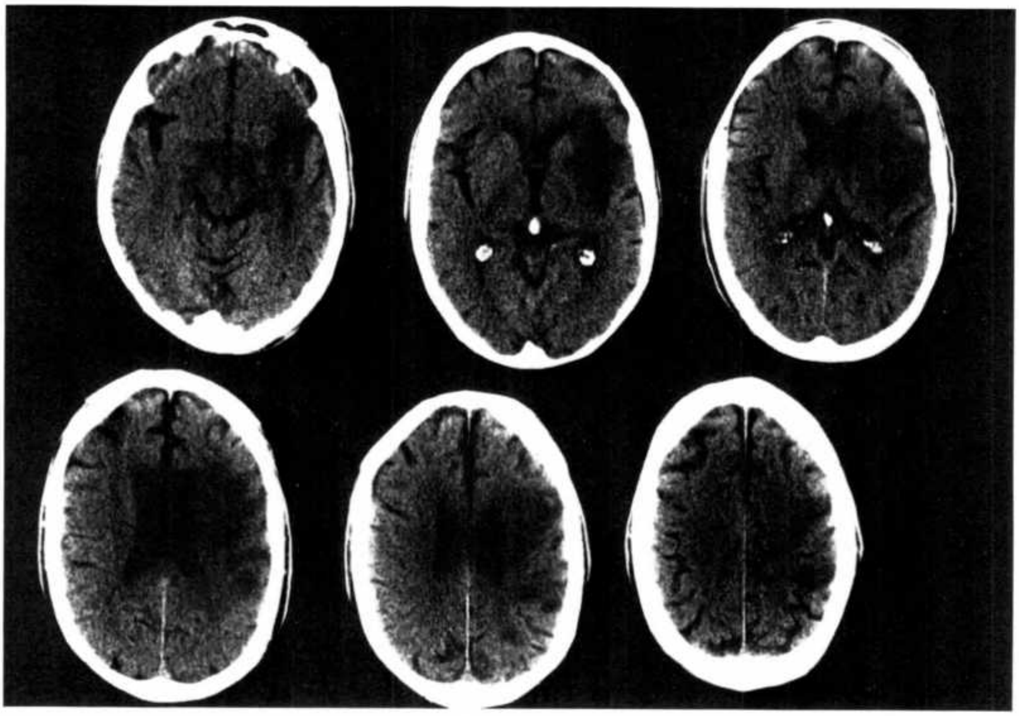

FIG. 2. CT scan performed 6 wks postonset in *Case 2*. There is a large infarct involving a portion of the territory of the left middle cerebral artery. The lesion involves the frontal operculum, lower motor cortex, anterior/medial insula and, in a patchy manner, sensory cortex and angular gyrus. There is also lesion in most of the putamen, the upper anterior limb internal capsule and the entire periventricular white matter.

The Boston Diagnostic Aphasia Examination (BDAE) was administered 2 and 8 wks postonset (Table 1). Initial language testing revealed mild naming difficulties and severe agraphia. Visual confrontation naming (90/114) and responsive naming (24/30) were compromised by prolonged response latencies and occasional semantic substitutions. By the time of discharge, naming skills had improved considerably (confrontation naming 114/114; responsive naming 30/30) Writing (with the left hand) was characterized by such poor orthography as to be an undecipherable scrawl. Spontaneous verbal output was generally fluent and grammatical, although utterances were terse and poorly elaborated. Auditory comprehension was adequate for word discrimination, yes/no reliability and conversational information. Reading was compromised by right neglect and poor attention.

Neuropsychological testing performed 6 wks after onset demonstrated deficits in attention, spatial function, insight and higher level cognitive tasks (Tables 2, 3). He also had severe deficits in memory, in part due to poor attention but also perhaps due to his long history of alcohol use. Visuospatial and basic visual perceptual deficits were striking. He had difficulty matching geometric designs (Benton Visual Form Discrimination Test 12/16) and his performance on the Benton Judgement of Line Test was severely impaired (8/30). Although there was no obvious neglect on line bisection, he was very slow to scan to the right and placed visual material in his left hemiattentional field. He could identify 12/14 map locations. Constructions were quite disorganized, poorly integrated with evidence of right-sided inattention. His scaled score on the block design subtest of the WAIS-R was 4 with broken configurations on the 3×3 designs (7 and 8).

Attention was modestly impaired. Although he was able to repeat 7 digits forwards and backwards (digit span subtest SS = 8), his visual span of attention was limited to 4 forwards and 3 backwards (Corsi Block). Auditory vigilance was only mildly deficient (Auditory As 25/26; Auditory XIs 24/26), but moderate motor impersistance was evident (4/7). Mental control was compromised by his inability to perform serial 3 addition (WMS 6/9). He demonstrated severe deficits in memory. On the CVLT—version for dementia—he was only able to learn 3/9 words over 5 presentations with evidence of severe proactive interference. Similarly, on the paired associates subtest of the WMS, he was only able to learn 3 of the easy pairs and none of the hard pairs over 3 presentations.

Insight was quite limited. He denied any deficits and was quite unconcerned and indifferent throughout his hospitalization. His affect was quite flat and he exhibited a impoverished range of emotional expression. On the Zung Self-Rating Depression Scale he did not endorse depressive symptoms and denied feeling distressed about his situation.

Case 3

This 65-yr-old man had abrupt onset of right-sided weakness and confusion. The initial evaluation demonstrated a normal ECG, left middle cerebral territory infarction on CT and left internal carotid artery occlusion by Doppler studies. Prolonged lethargic confusion delayed transfer for rehabilitation for 3 wks. We examined him at 4 and 9 wks postonset. He was exclusively right handed for all unimanual tasks and there was no family history of left handedness. There was no personal or family history of early life brain injury, seizures or learning disability. He was a high school graduate.

Neurological examination demonstrated a severe spastic right hemiplegia and sensory loss, and severe right visual neglect with no definite visual field loss. There was marked hypophonia, but no dysarthria. Three independent raters judged him to have marked reduction in affective prosody. There was severe buccofacial and limb apraxia. He was unable to produce any melody in song. Follow-up examination at 9 wks postonset demonstrated no substantial changes.

A CT cranial scan performed 5 wks after onset demonstrated left cerebral infarction (fig. 3). There was only patchy deep frontal lesion and patchy putaminal lesion, but extensive damage to the parietal lobe, the entire periventricular white matter, and the temporal isthmus (Alexander *et al.*, 1987). There was no right hemisphere lesion. The cerebral asymmetries were typical posteriorly but atypical anteriorly, that is, the left frontal region was longer than the right, the reverse of the usual pattern in right-handed men (Koff *et al.*, 1986).

Formal language examination using subtests of the BDAE demonstrated substantial deficits in all language modalities (Table 1). Performance was compromised by poor attention, distractability, severe perseveration and confusion. Errors in spontaneous speech were confabulatory, tangential and perseverative. Naming errors were perseverative and perceptual. For example, on the Boston Naming Test (BNT), he called a 'pelican', a 'trumpet'; a 'stethoscope', a 'trumpet igloo'; and a 'pretzel', a 'fence'. Auditory comprehension was variable in that errors seemed to be due to attentional lapses, perseveration, and difficulty getting into set. Comprehension during information conversation was adequate. On the Token Test, he was only able to respond accurately to 1/16 items, but severe right neglect and perservation impaired his performance.

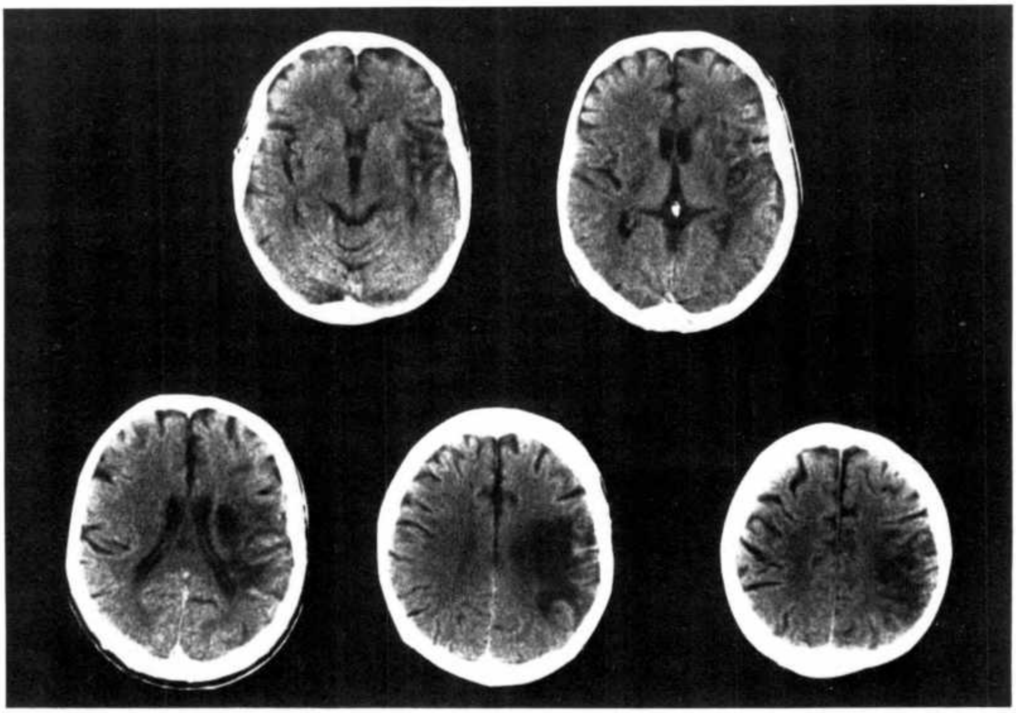

FIG. 3. CT scan performed 5 wks postonset in *Case 3*. There is infarction in a portion of the territory of the left middle cerebral artery. Primary damage is to the entire periventricular white matter and corona radiata and the cortex and subcortical white matter of the parietal lobe, reaching to the superior parietal lobule. There is patchy damage to the entire insula.

There was severe agraphia manifested as poor orthography and perseveration. Reading aloud and reading comprehension were compromised by severe right-sided neglect and distractability.

Neuropsychological testing performed 7 wks after onset (Tables 2, 3) demonstrated profound disturbances in attention, spatial function, insight, higher level cognitive tasks and memory. Much of the disturbance was due to his severe attentional problems.

Severe visuospatial and visuoperceptual deficits were evident. He was unable to match many geometric designs on the Benton Visual Form Discrimination Test (0/16) and his performance on the Benton Judgement of Line Test was severely impaired (0/30). He was unable to identify any map locations (0/14). Line bisection was compromised by severe right neglect. Constructions were segmented, overdrawn, with loss of overall configuration of target.

Attention was also profoundly impaired. He was distractable and perseverative throughout the evaluation. On the digit span subtest of the WAIS-R, he was able to repeat 6 digits forwards, but only 2 backwards (SS = 4). Visual span of attention was limited to 3 forwards and 2 backwards (Corsi Block). His performance on auditory vigilance tasks was impaired (Auditory As 20/26 with 7 false positives). Severe motor impersistence was evident (0/7). He was disorientated for place and time. He was unable to recall any information from the logical memory and visual reproduction subtests of the WMS (0/23 and 0/14 respectively). Insight was severely impaired, he demonstrated no concern or interest, and his affect was extremely flat.

Case 4

This 30-yr-old man collapsed abruptly. Emergency evaluation demonstrated obtundation, right hemiplegia and evolving left third nerve palsy. CT revealed a large left temporal intracerebral haemorrhage. At emergency craniotomy the clot was evacuated and a left middle cerebral artery aneurysm was clipped. In the postoperative period there was gradual improvement in alertness, and he was transferred for rehabilitation after 5 wks. We examined him at 5 and 11 wks after onset. He was exclusively right handed for all unimanual tasks (writing, tool usage, and toiletry tasks) except throwing; he batted left handedly. His mother and 2 of his 7 siblings were left handed; he had no children. In his previous medical history he had a respiratory arrest at 18 months in the context of an upper respiratory infection. He underwent an emergency tracheostomy, but the family was unaware of any neurological sequelae. His developmental milestones were normal, but he was a poor student. He had a school evaluation and was labelled as learning disabled. He attended special classes and graduated from high school in a vocational program. He has worked in a stockroom position for 12 yrs. He had a history of moderate alcohol abuse.

Neurological examination demonstrated a dense spastic right hemiplegia, right-sided sensory loss and a right hemianopia. He had a partial left third nerve palsy. Praxis was normal. He had moderate hypophonia, but no dysarthria. By our clinical judgement he had marked affective dysprosody. He was unable to produce melody in song.

CT performed 6 wks after onset demonstrated a large left-sided infarct (fig. 4). Damage was similar to that in Case 3 although with less parietal injury. There was no right-sided lesion, but the ventricles were large for his age. Cerebral asymmetries were typical (Koff *et al.*, 1986).

Language evaluation using subtests of the BDAE showed no definite primary language deficits except profound agraphia (Table 1). Spontaneous speech was fluent and grammatical with no paraphasic errors. Decreased performance on formal measures of auditory comprehension was secondary to poor attention, reduced recall, and impulsivity. Right-sided neglect was initially so severe that reading material could not be placed in a manner that he could read text; reading vertically presented material in the far left visual field was normal. At 11 wks after onset, right-sided pseudoalexia was much less marked. He was able to read sentences with external visual cues.

Neuropsychological assessment performed 6 wks after onset demonstrated severe impairments in attention, spatial function, particularly severe right-sided neglect, insight and higher level cognitive tasks (Tables 2, 3). Visuospatial and visuoperceptual abilities were severely impaired. He had difficulty matching simple and complex geometric designs (Benton Visual Form Discrimination Test 5/16) and his performance on

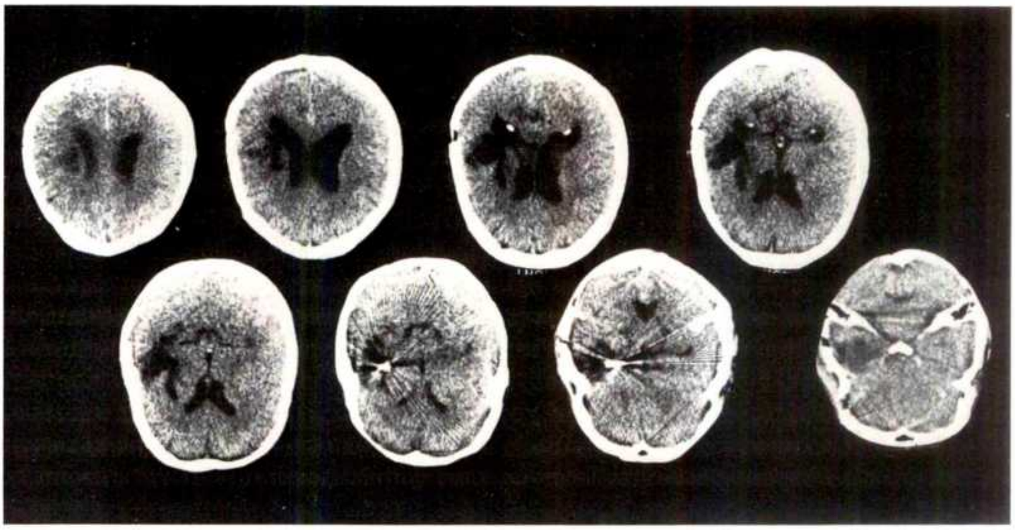

FIG. 4. CT scan performed 6 wks postonset in *Case 4*. The surgical clip on the left middle cerebral artery aneurysm is seen. Hypodensity, reflecting a combination of the surgical resection and infarction, is noted in the anterior temporal lobe, superior temporal gyrus and inferior parietal lobule. There is also a lesion involving the putamen, the posterior two-thirds of the periventricular white matter and the entire deep temporal white matter and temporal isthmus.

TABLE 3 SUPPLEMENTARY NEUROPSYCHOLOGICAL TESTING

	Case 1	*Case 2*	*Case 3*	*Case 4*
WAIS-R				
VIQ	109	77	62	74
PIQ	87	Prorated 70	Unattainable	Unattainable
FSIQ	98	73	Unattainable	Unattainable
Information	13	8	1	6
Vocabulary	14	5	1	6
Arithmetic	6	6	7	3
Comprehension	11	3	1	7
Similarities	11	1	1	6
Picture completion	5	2	–	5
Picture arrangement	5	4	–	Never in set
Block design	7 broken config. no. 7	4 broken config.	−1 broken config.	
Object assembly	7 unable to assem hand		2	−1
Digit symbol	5	8	–	4
Memory				
WMS-MQ	118	71	64	64
Information	5/6	4/6	3/6	5/6
Orientation	4/5	2/5	3/5	4/5
Logical memory	10/23	6/23	0/23	7.5/23
Mental control	5/9	6/9	6/9	2/9
Visual reproduction	7/14	0/14	0/14	1/14
Paired associates	18/18	2.5/18 (1,0)(1,0)(3,0)	4/18 (3,0)(4,0)(2,0)	8/18 (3,1)(5,1)(5,0)
California Learning Test (version for dementia)				
Monday list	5,7,8,9,9-9/9 total	5,5,3,4,3-3/9 total	2,3,3,6,4-4/9 total	5,5,5,6,6-6/9 total
Tuesday list	4/9	4/9	5/9	3/9
Monday immediate	9/9	0/9	0/9	2/9
Monday delay	7/9	0/9	0/9	4/9
Higher level cognitive functions				
Wisconsin	0/6, 74% persev	–	–	
Raven's	−25 percentile	Never in set	<10 percentile	

the Judgement of Line Test (0/30) was compromised by severe right neglect and difficulty perceiving the angular placement of the stimulus array. There was marked right neglect on Line Bisection (6/19 correct) and visual cancellation tasks. Drawings from copy of simple geometric designs were poorly organized and integrated with marked segmentation and loss of overall configuration He could only identify 1/14 map locations with evidence of severe right neglect.

Auditory attention as measured by the digit span subtest of the WAIS-R was 6 forwards and 2 backwards (SS = 4). His result on the Corsi Block Test was only 2 forwards and 2 backwards with marked right-sided neglect. On Auditory As, he was only able to respond accurately to 22/26 targets and on XIs only 16/26. On the mental control subtest of the WMS (2/9), he lost set on serial 3 addition and was unable to recite the alphabet appropriately. He was unable to sustain any motor movements appropriately on motor impersistence tasks (0/7).

Affect was extremely flat with a limited range of emotional expression. Insight was poor with no appreciation of his deficits or resulting physical or cognitive limitations. On the Zung Self-Rating Depression Scale he denied any symptoms indicative of depression and saw no reason why he would not be able to function as he had before his admission

DISCUSSION

Analysis of the lateralization and localization of functions

Language. Despite ambiguous results on formal tests of language (*see below*), our patients had no or minimal aphasia (excluding agraphia and right pseudoalexia). Recent CT investigations by Naeser (1983) have reported specific relationships between lesion size, lesion location and aphasia syndromes in patients with unilateral left hemisphere brain damage. Highly significant correlations were reported between lesion size and severity of aphasia as well as lesion site and 'fluency' of the speech output and type of aphasia. According to those studies and to 'classical' aphasiology (Benson and Geschwind, 1989), Cases 1 and 2 might have been expected to present with severe nonfluent aphasia and Cases 3 and 4 with Wernicke's aphasia.

In our cases there was no aphasia, or at most there were only limited deficits, despite extensive damage to perisylvian regions, superficially and deep. The absence of language representation in the left hemisphere suggests that these patients probably would have presented with crossed aphasia had they suffered right hemisphere lesions. Except for writing, the instrumentalities of language were not involved, and so the specific right hemisphere localizations of language functions are unknown. Agraphia (with the nonpreferred hand) was severe in 3 patients (Cases 2–4) and appeared to be due to the combined effect of spatial and attentional deficits upon orthography. In Cases 2 and 3, there may have also been apraxic agraphia (Roeltgen and Heilman, 1983). Confrontation naming deficits were observed in the same 3 patients (Cases 2–4) and again seemed to reflect the interaction of impaired global attention, perceptual deficits and even specifically right-sided inattention. Auditory comprehension deficits (Cases 3, 4) and bizarre language content (Case 3) were unequivocally related to attentional limitatations (*see below*).

Praxis. Cases 2 and 3 demonstrated severe buccofacial and limb apraxia typical of right handers with left hemisphere lesions. The finding of limb and buccofacial apraxia without a disorder of speech and language is rare, but supports previous reports of the independence of these two functions (Heilman *et al.*, 1973, 1974; Selnes *et al.*, 1982; Junqué *et al.*, 1986; Judd, 1989). In both these cases the intrahemispheric lesion location fits traditional models of lesion localization for buccofacial (Tognola and Vignolo, 1980) and limb (Kertesz and Ferro, 1984) apraxia. For Cases 1 and 4, the lesion distribution in the left hemisphere should have produced (particularly for Case 1) severe and persistent limb and buccofacial apraxia (Kertesz and Ferro, 1984) but did not. Thus, in these 2 cases, 1 of whom had first degree left-handed relatives and both of whom had histories of mixed handedness, limb praxis was apparently mediated by the right hemisphere. This suggests that right-handers with histories of minor mixed handedness or right-handers with left-handed first degree relatives may have higher level motor control lateralized to either (or both) hemispheres. In this sense cerebral mediation of learned movement may be more related to handedness or family history of handedness than language or even production of speech. Perhaps this is the meaning of 'handedness'—the hemisphere in which lateralized limb motor learning resides? Furthermore, these cases suggest that the hemisphere responsible for buccofacial skills may or may not be the one that is responsible for handedness or language or speech (Mani and Levine, 1987), at least in right-handers.

Visuospatial functions. Cases 2, 3 and 4 had severe visuospatial deficits and right-sided neglect. Their copies of simple and complex geometric designs reflected a segmented approach with loss of the overall configuration. This type of visuospatial impairment is usually found in patients with right hemisphere dysfunction (Goodglass and Kaplan, 1979; Milberg *et al.*, 1986; Walsh, 1987). The profile of clinical deficits would fit the intrahemispheric lesion distribution had the lesion involved the right hemisphere (Heilman *et al.*, 1983; Kertesz, 1983). Case 1 had milder visuospatial deficits and no obvious neglect on line bisection and visual cancellation tasks. Her constructions were compromised by overdrawing and disorganization which is more typical of patients with right anterior brain system dysfunction (Walsh, 1987). Her lesion destroyed the entire left frontoparietal operculum as well as anterior subcortical structures which would probably be consistent with the intrahemispheric relationships had she suffered a right hemisphere stroke.

Attention. Impaired attention was evident in all patients. Case 3 suffered from a persistent confusional state (profound attentional disturbance); this has been described previously for infarctions of the right middle cerebral artery territory (Mesulam *et al.*, 1976). The other cases had impaired attention, manifested as motor impersistence, poor mental control and frequent loss of cognitive set. Impairments in attention and concentration (Mesulam, 1981), and motor impersistence (Kertesz *et al.*, 1985) are usually associated with right hemisphere injury, but in these cases were produced after unilateral left hemisphere dysfunction. The intrahemispheric relationships are probably consistent with what would have been expected if the lesion affected the right hemisphere alone. Patients with the more global attentional disturbances and right hemiattentional deficits (Cases 3, 4) had the most extensive parietal and deep parietal lesions (Mesulam, 1981). The more anterior lesion cases (1, 2) had milder, but definite, attentional deficits with accompanying motor impersistence (Kertesz *et al.*, 1985).

Affect and emotion. Each of these 4 patients had poor affective prosody in spontaneous speech as well as an overall reduction in emotional expression. These functions may be impaired after right hemisphere damage, particularly damage to the posterior frontal lobe or its descending pathways (Ross, 1981). We did not specifically test phonemic stress or emphatic stress and linguistic prosodic deficits at the sentence level and, as such, it remains uncertain if the same right hemisphere lesion also disturbed propositional nonaffective prosody (Weintraub *et al.*, 1981; Cooper *et al.*, 1984; Shapiro and Danly, 1985; Behrens, 1989). Despite these limitations in acoustic analysis, there was absolutely no articulatory deficit in any of our patients. Melody in singing, which may rely uniquely on right hemisphere integrity and may use the same motor system as affective prosody (Gordon and Bogen, 1974; Sidtis and Volpe, 1988), was also impaired.

All 4 patients minimized or denied the extent of their deficits or disabilities. They specifically denied depression, and a self-rating of mood indicated no distress/concern. Unconcern and denial characterize the indifference reaction which has traditionally been associated with right hemisphere damage, not further localized (Weinstein and Kahn, 1955; Gainotti, 1972).

Issues that confound analysis of 'exceptions'

There is a remarkable discrepancy in the prevalences of reports about 'exceptions' of aphasia and 'exceptions' of everything else. Between 1 and 2% of all right-handed aphasics are right hemisphere dominant for language (Gloning *et al.*, 1969), but there are no reported estimates of other reversed lateralities. By comparison, we recently reviewed 34 well-described cases of crossed aphasia with well-defined local lesions (Alexander *et al.*, 1989), but could find only 3 cases of other lateralization anomalies (Selnes *et al.*, 1982; Junqué *et al.*, 1986; Judd, 1989). Population studies defining the incidence of reversed lateralities appear necessary to resolve this question. Without such studies the reason for this discrepancy in incidence reports is unknown. It could be due to a bias of interest toward aphasia or to a true difference in the prevalence and thus the mechanisms of the two events. Geschwind and Galaburda (1985), for instance, have suggested that certain aspects of right hemisphere dominance rarely shift to the left because the right hemisphere develops earlier in utero; interfering factors would then be less likely to disturb its development.

In our earlier report (Alexander *et al.*, 1989), we also reviewed the data available about the prevalence of 'exceptions' to standard aphasia theory. There are numerous reasons why these data are hard to interpret. First, there is no universally accepted theory as to which aphasias are 'standard'. Secondly, there is no uniformly applied operational definition of aphasia profiles from which to construct 'standards'. Thirdly, 'standard' aphasias are usually reported as syndromes and, whatever the merits of syndromes for clinical diagnosis, they are manifestly too complexly determined to be the elemental biological building blocks of aphasia. Fourthly, more careful analysis may transform a large number of 'exceptions' into 'standards'. Basso *et al.* (1985*b*), for instance, suggested that 17% of all unilateral left hemisphere cases had unexpected aphasia profiles given their lesion distributions. We proposed that finer grained aphasia diagnoses and more detailed consideration of subcortical anatomy might make that prevalence figure even smaller (Alexander *et al.*, 1989).

Another factor that potentially confounds the interpretation of deficits in our patients is the difficulty in identifying defective instrumental cognitive operations in the presence of impairments of attention. Profound deficits in attention can confound results obtained by formal quantitative language assessment in the absence of a primary aphasic disorder. For example, Levin *et al.* (1976) reported that naming, word association, and sentence comprehension were dramatically decreased in severely head-injured patients. This decline could be attributed to poor attention rather than to specific linguistic deficits. Chédru and Geschwind (1972) described agraphia with orthographic and spatial deficits in normal subjects secondary to anaesthesia-induced confusion. Quantitative methods of language assessment are limited with these clinical populations by the absence of a suitable metric to differentiate task-specific from non-task-specific mechanisms of poor test performance. Our patients with severe right hemisphere syndromes had intact verbal abilities, but the demands made by formal language assessments on their impaired attentional systems compromised their formal language scores (Table 1).

Theoretical issues

Despite these confounding issues and the inevitably small numbers of cases analysed, anomalous cases may illuminate the phenomenology of all 'exceptions' and the basic mechanisms of lateralization. The organization of brain functions in crossed aphasia and probably in some left handers clearly deviates from the model of cerebral organization developed from the study of standard right handers. In crossed aphasics (and left handers) supplementary models for the organization and relationship of lateralized functions have been suggested. All these models have incorporated language, visuospatial skills and praxis. In some, there has been little further differentiation of subfunctions (Kreindler *et al.*, 1966; Bradshaw and Nettleton, 1981; Watson and Heilman, 1983). In others, there has been consideration of fractionated capacities, such as between written and spoken language (Hécaen and Sauguet, 1971; Joanette *et al.*, 1982; Basso *et al.*, 1985*a*), buccofacial and limb apraxia (Basso *et al.*, 1985*a*; Mani and Levine, 1987), differential qualities of visuospatial functions (Delis *et al.*, 1983; Basso *et al.*, 1985*a*), and affective and emotional functions (Henderson, 1983; Ross *et al.*, 1989). Most models of anomalous lateralization have only considered the overall laterality of the lesion, not its intrahemispheric location. Since development of CT, however, some reports have specified more precise lesion sites (Joanette *et al.*, 1982; Henderson, 1983; Basso *et al.*, 1985*a*). Following an expanded model of cerebral organization that includes all of the above separable differentiated functions plus global attention and intrahemispheric lesion location, we proposed that lateralization and localization of cerebral functions in crossed aphasia could be characterized as mirror image or as one of several anomalous types (Alexander *et al.*, 1989).

The present study extends our previous report and confirms that the identification of anomalous profiles must include consideration of more than overall language and overall visuospatial functions or simply laterality of lesion. Many of the so-called 'exceptions' may reflect primary biological variations in how cognitive functions are organized within and between the hemispheres. Crossed aphasia and the type of totally anomalous cases reported here and by Judd (1989) are the most 'exceptional' of the 'exceptions', and they may account for a sizeable minority of all exceptions. Analysis of the individual cognitive operations, even at the gross clinical level, may identify other, less blatant, patterns of exception. When there is a coherent theoretical account for 'exceptions', the standard theories will be more clearly scrutinized.

The biological mechanisms which determine the interaction of these cerebral functions as they develop are not known. Geschwind and Galaburda (1985) suggest they were related to an interaction between genetic, hormonal, and environmental influences. They cannot be random as the distribution of deficit profiles for language and spatial impairments after unilateral brain lesions are not random. That commonly associated functions can be entirely dissociated in some patients indicates that there are no obligatory links in the development of those functions. For instance, theories that language is linked to the hemisphere that masters sequential motor learning (Kimura and Archibald, 1974) are inconsistent with those patients who present with a complete dissociation of praxis and language functions. In fact, the traditional 'right hemisphere' functions—global attention, configurational spatial capacities, and certain aspects of emotional functioning—seem more robustly linked together than language and praxis or even than written and spoken language are to each other (Hécaen and Sauguet, 1971; Alexander *et al.*, 1989). The reason for the higher incidence of reports and crossed aphasia than of other crossed functions may be that language is the most flexibly lateralized function. Furthermore, whatever the lateralization, the localizations of language operations may also be the most flexibly organized of all cognitive operations, and thus the 'exceptions' (Basso *et al.*, 1985*b*).

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(Received September 19, 1989. Revised February 13, 1990. Accepted March 6, 1990)

Brain (1991), **114**, 263–280

SHORT-TERM MEMORY AND SENTENCE COMPREHENSION

AN INVESTIGATION OF A PATIENT WITH CROSSED APHASIA

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SUMMARY

The relationship between short-term memory impairment and sentence comprehension is explored in a right-handed patient with a focal temporoparietal lesion of the right hemisphere. The general clinical profile, as well as characteristics of the patient's immediate memory for word lists, suggests the occurrence of a 'mirror image' crossed aphasia. Detailed analysis of the patient's ability to repeat and to comprehend sentences, however, indicates some important differences between this case and previously reported patients with short-term memory impairment. It is suggested that these differences, which may be related to an unusual pattern of neuroanatomical organization of cognitive functions, involve symptom dissociations with implications for models of normal language/memory interactions.

INTRODUCTION

'Crossed aphasia' is the term used to describe the occurrence of aphasia following unilateral cerebral involvement of the hemisphere ipsilateral to the patient's dominant hand (Bramwell, 1899). In part because it is a relatively rare occurrence, crossed aphasia has attracted considerable interest from neurologists in recent years. A number of well described cases have been reported, and these are the subject of a recent comprehensive review by Alexander *et al.* (1989) (*see also* Castro-Caldas *et al.*, 1987). Two issues have been the primary focus of interest in crossed aphasia. First, language performance and CT scan information (where available) have been used to address the question as to whether patients with crossed aphasia demonstrate a 'mirror image' of the symptom/lesion locus correlations found in standard aphasia, or exhibit unique patterns of lesion/deficit relations. Alexander *et al.* (1989) have argued persuasively in response to this question that both 'mirror image' cases and quite anomalous patterns can occur in crossed aphasia.

The second issue that has been addressed in analyses of crossed aphasia concerns the occurrence of dissociations among neuropsychological symptoms that are frequently found to accompany language disturbance in left-damaged, right-handed individuals. Such dissociations suggest considerable flexibility in the development of cerebral lateralization, but they are also revealing of important functional dissociations. The best documented example along these lines is the frequent dissociation of limb apraxia from

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crossed aphasia (Brown and Wilson, 1973; Castro-Caldas *et al.*, 1987). This dissociation undermines the claim that both limb apraxia and aphasia result from a single higher order deficit of symbolization (Goldstein, 1948), as well as the hypothesis that language and praxis are evolutionarily linked to the same neuroanatomical sites in the left hemisphere (Kimura, 1979).

The demonstration of such functional dissociations suggests that cases of crossed aphasia may be the source of detailed information about the normal functional organization of the cognitive system by revealing other, perhaps more subtle, symptom dissociations. Recent cognitive neuropsychological investigations of language have relied on the interpretation of symptom associations and dissociations as sources of support for functional models of the cognitive system (Coltheart, 1985; Shallice, 1979; Ellis and Young, 1988). In fact, the *dissociation* of two symptoms that are frequently found to co-occur has been taken as evidence that a particular functional model has been falsified (e.g., Caramazza and Berndt, 1985). However, the finding that two symptoms are always found to be *associated* does not carry the same theoretical weight (*but see* Caramazza, 1986). Although the consistent association of two symptoms may indeed reflect their common functional basis, such co-occurrence might also signal neuroanatomical accident: the symptoms may arise from two separate functional deficits that happen to be related to adjacent neural tissue (*see* Ellis and Young, 1988, Chapter 1, for arguments). The frequent co-occurrence of aphasia and limb apraxia in right handed patients may reflect such 'neuroanatomical proximity'. Thus the unusual examples of lateralization of function found in cases of crossed aphasia may present opportunities for the discovery of symptom dissociations that will help to constrain functional models of language.

This report addresses both of the issues that have been the focus of recent studies of patients with crossed aphasia through examination of the symptoms of a right handed patient with a right hemisphere lesion who superficially presents a 'mirror image' conduction aphasia. On closer examination, however, her symptoms appear to differ in important ways from the symptoms of other similar patients and, in fact, demonstrate dissociations among frequently co-occurring symptoms.

Working memory and sentence comprehension

Since the groundbreaking description by Warrington and Shallice (1969) of a patient with selectively impaired short-term verbal memory, a variety of studies have been carried out with brain-damaged patients who demonstrate apparently similar deficits. Often (but not always) classified as conduction aphasics, these patients typically demonstrate relatively fluent spontaneous speech, relatively good auditory comprehension on clinical testing, and a striking reduction of immediate repetition span. Several generalizations have emerged from these case studies: (1) unlike normal subjects, these patients show a repetition span for auditory material that is somewhat shorter than their span for visual material; (2) span tasks that do not require speech production (pointing and matching span) yield results comparable with standard repetition span tasks; and (3) auditory single-word comprehension is well preserved, but sentence comprehension is frequently impaired for lengthy and/or syntactically complex sentences, or those in which comprehension depends on the interpretation of serial order.

Until very recently, it could be said that all of the well-studied patients with short-term memory deficit demonstrated some impairment of the ability to comprehend sentences.

The co-occurrence of these two symptoms has been widely interpreted as a reflection of the important role of short-term memory in the comprehension process (Saffran and Marin, 1975; Caramazza *et al.*, 1981; Vallar and Baddeley, 1984*b*). In the past several years, however, dissociations between the two symptoms have been reported such that individuals with severely reduced immediate repetition span have proven capable of comprehending a range of syntactically complex sentences (Butterworth *et al.*, 1986; McCarthy and Warrington, 1987*b*; Martin, 1987). These reports have led to the suggestion that the memory system responsible for the verbatim repetition of word lists may not be the same as the 'working memory' system that subserves language processing. McCarthy and Warrington (1987*b*), for example, have proposed that there are separable memory systems for dynamic 'on-line' sentence processing and for the veridical storage of phonological information. Dissociations result from impairment to one system, with sparing of the other. Similar alternative proposals have avoided this kind of dichotomy, and have proposed instead that working memory should be conceived of as a multicapacity system for the transient storage of representations of several distinct codes (auditory, phonological, semantic, syntactic) that are generated during language processing (Monsell, 1984; Saffran, 1990). Sentence materials generate the largest number of potential representational codes that can serve as a basis for retention; thus, sentence-length material may be retained well enough for comprehension (and even for repetition) when word lists (with fewer representational codes generated) are not retained (*see also* Berndt, 1988). It must be noted, however, that this latter interpretation has difficulty accounting for the rare cases (such as Case 3, McCarthy and Warrington, 1987*a*) in which word list repetition is superior to sentence repetition.

These kinds of considerations have resulted in a new emphasis on examination of the basic language processing capacities of patients with suspected memory impairment in an attempt to characterize the status of the various representational codes that must be generated and maintained for adequate sentence processing. Several studies have investigated patients' generation of phonological (Allport, 1984; Friedrich *et al.*, 1985), articulatory (Martin, 1987) and lexical codes (Saffran and N. Martin, 1990; Martin *et al.*, 1991) as sources of information that can be retained for repetition and comprehension. These investigations have demonstrated that selective deficits in each of these areas may have discernible effects on patients' repetition spans, but that their effects on comprehension vary widely. This report proceeds in the same vein, by attempting to characterize the adequacy of the codes that the patient generates in an attempt to understand her apparent inability to retain them.

CASE REPORT

E.D.E. was a 56-yr-old housewife in August 1982, when she developed an acute right cerebrovascular lesion. The initial symptoms included disordered speech, agitation with sudden changes of mood, and left arm drift. A CT scan on admission showed right temporoparietal infarction; cerebral angiography revealed tight stenosis of the proximal right carotid artery. An endarterectomy was performed in September 1982. E.D.E. improved gradually over the next several months, although language comprehension disorder and labile affect persisted. She was admitted to the University of Maryland Hospital in June 1983, for treatment of jacksonian seizures that began in the left hand and progressed to become generalized.

On admission to this service (10 months postonset) E.D.E. demonstrated agitation, distractability and unusual anxiety. She experienced abrupt changes of mood, even within a few minutes, from agitation to lethargy, and from elation to despair. She complained of poor memory. Speech was fluent with occasional phonemic paraphasias. Naming of objects and repetition of phrases and sentences were moderately impaired. Auditory comprehension was also impaired, with poor execution of two-step commands. Comprehension of written words was better than auditory, with correct performance of written two-step commands. Spontaneous writing was severely impaired, even for her own name, although she could copy written words and sentences easily in both cursive writing and print. Simple addition and subtraction were completed correctly, but she could not perform multiplication or division.

No motor involvement (i.e., limb apraxia, hemiparesis) was found; there was evidence of slight neglect on the left side to bilateral simultaneous tactile stimulation. Visual fields were full and intact, with no evidence of neglect to bilateral visual stimulation. She easily copied accurately both symmetric and asymmetric line drawings. Pure tone audiometry showed normal hearing within the speech frequencies, with a mild high frequency loss. Electroencephalography showed frequent 1.5 − 3 s 'pseudoperiodic' activity in the right anterior quadrant. A CT scan (*see* fig. 1) showed a lesion involving the right superior temporal gyrus, supramarginal gyrus and part of the middle temporal gyrus. No damage was seen in the left

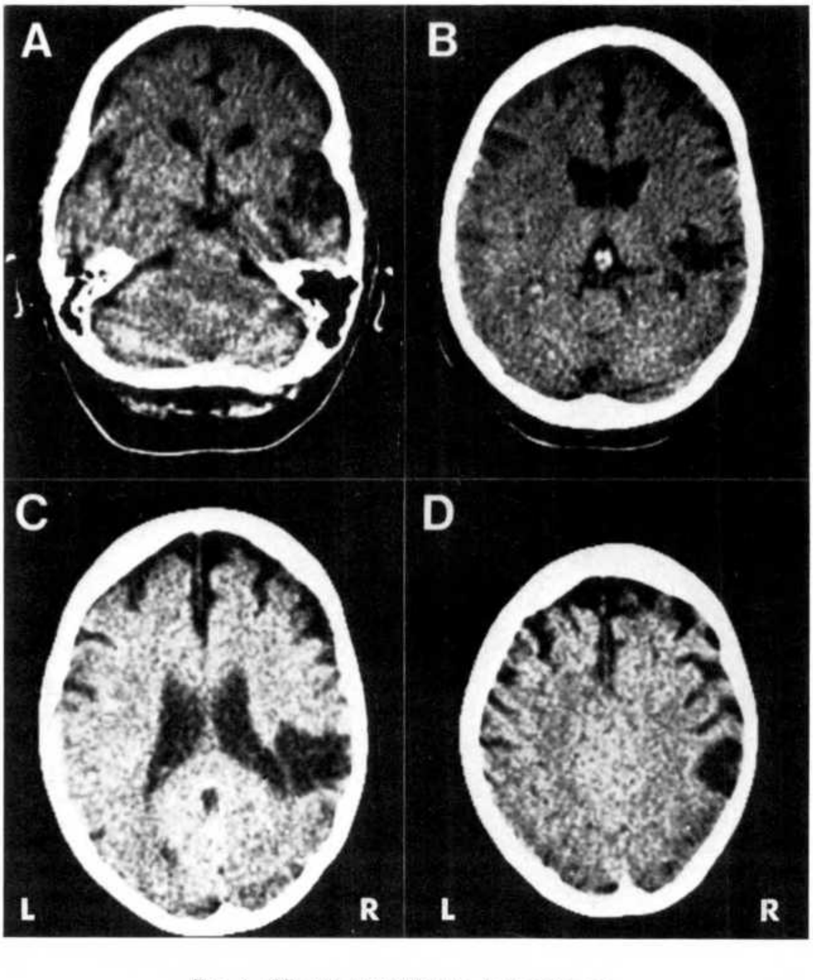

FIG. 1. CT scan approximately 1 yr postonset.

hemisphere. She was discharged home on phenobarbital 30 mg thrice daily and has had no seizures since discharge.

E.D.E. is a native speaker of American English who completed high school and formerly worked as a secretary. She reports, and her husband confirms, that she has always been right handed, with no history of left handedness in the immediate family. She scored as a dextral on the Harris Test of Lateral Dominance (Harris, 1955).

At the time of her discharge, E.D.E. consented to enter into a research study, which she was told would focus on her memory and comprehension problems. She was seen intermittently over the next several years, during which time her repetition span and sentence comprehension performance improved slightly, as noted below. Reading and writing, in contrast, improved more dramatically and were functional in 1984. Her affective disorder has also resolved; she currently demonstrates appropriate affect, with no abnormal lability. An MRI scan was obtained in December 1987. As shown in fig. 2, the extent of the lesion is similar to that seen on the earlier CT scan; the small deep anterior centrum semiovale lesion seen on the right on the T_2-weighted image in fig. 2 was not seen on the T_1 image. Fig. 3 shows the posterior extent of the lesion. The left hemisphere appears to be entirely intact on MRI.

Clinical assessment

Clinical assessment of cognitive functions was carried out at the beginning of this study, approximately 1 yr following the right cerebrovascular lesion, and 3 months following the onset of seizure activity.

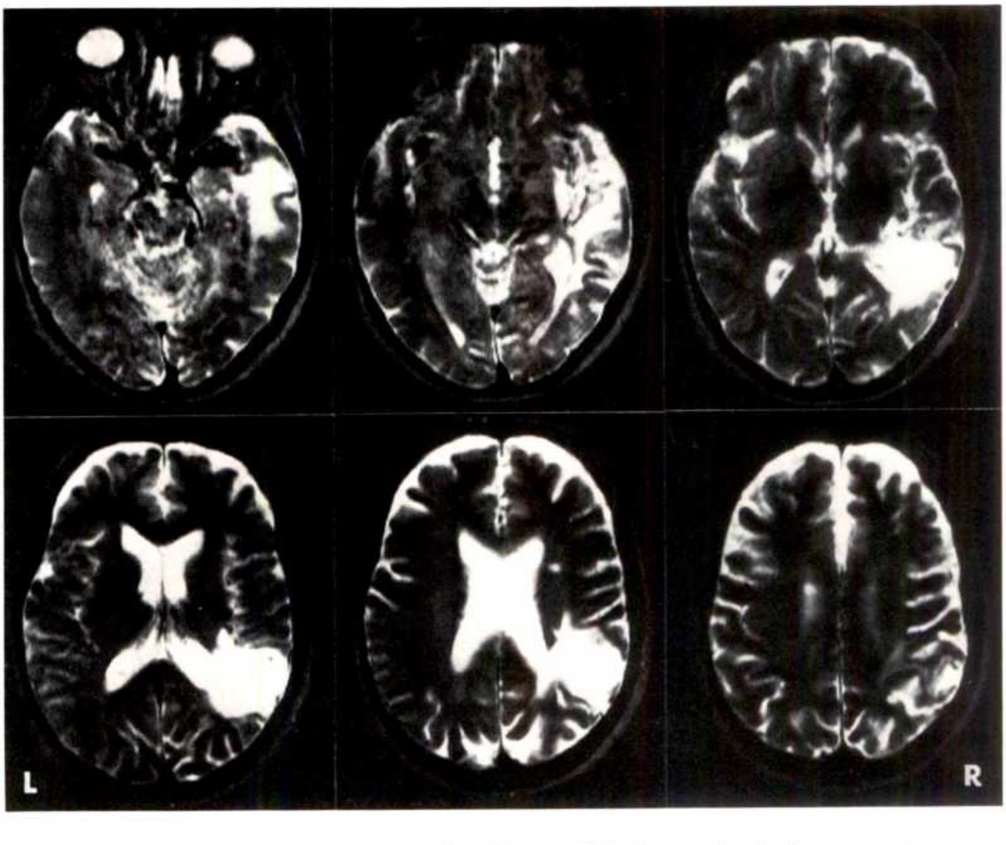

FIG. 2. Magnetic resonance scan (T_2-weighted image) obtained approximately 4 yrs postonset.

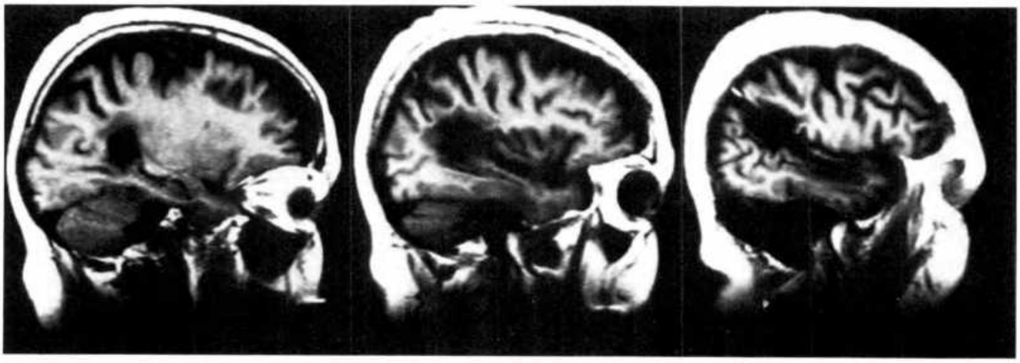

FIG. 3. Magnetic resonance scan, lateral view of right hemisphere (T_1-weighted image), approximately 4 yrs postonset.

Language

E.D.E. generally matched the profile of a conduction aphasic on the Boston Diagnostic Aphasia Examination (Goodglass and Kaplan, 1972) in that she produced fluent, sometimes paraphasic speech and poor repetition of sentences ($z = -0.5$). However, auditory comprehension was below the level that should be found in that class ($z = -1.5$). Commands (+2/15) and complex material (+1/12) were particularly poorly understood, but comprehension of single words was good except for body parts (+2/20). Confrontation naming and oral reading were relatively spared ($z = +1$ for both subtests). Reading comprehension was good for single words (+18/18), although somewhat disturbed for sentences and paragraphs (+7/10). Comprehension of oral spelling was very poor (+1/8). Copying a printed sentence into cursive script was perfect, but E.D.E. could not write the alphabet, letters or numbers to dictation; 5 of 11 primer-level words were written correctly to dictation.

Further assessment of reading and writing skills showed marked improvement in the first 6 months of follow-up. By January 1984, oral reading of single words was excellent for high imageable words (27/28) and fair for low imageable words (17/28), with no particular difficulty reading inflected words (13/15) or functors (43/43) (all materials from frequency-matched lists of M. Coltheart, unpublished). Nonword reading stabilized at approximately 75% correct for single syllable nonwords, with most errors being lexicalizations. Comprehension of single printed words was excellent in category sorting, picture-word matching and Coltheart's high imageable synonyms task.

Writing words to dictation improved rapidly but continues to show a slight effect of imageability. Writing of nonwords to dictation remains poor, but shows some retained information about phoneme-to-grapheme conversion. Written naming (34/60) was somewhat worse than oral naming (43/60) on the Boston Naming Test (Kaplan *et al.*, 1983), with 46% of written errors classed as letter omissions or reversals (e.g., *cactus* → 'catcus'; *sphynx* → 'sphyx'). Verbal naming errors were phonemic paraphasias (e.g., volcano → 'kɔ/veno/' (47% of errors); failures to respond (30%); and semantic paraphasias (*rhinoceros* → 'bull') (23%). The Peabody Picture Vocabulary Test (Dunn, 1965) yielded a raw score of 115 (ceiling = 140), indicating moderately good auditory comprehension of single words. Comprehension of sentences was very poor as assessed by the Token Test (De Renzi and Vignolo, 1962), (+6/10, part I; +2/10, part II; 0/10, part III). Although she was given a repetition of all items failed, E.D.E.'s most frequent response was 'I forget it'.

Memory

Remote memory. E.D.E.'s complaints of poor memory focused on her inability to remember people's names and telephone numbers. In our earliest interactions with her, she could relate in considerable detail the specifics of her illness and of her daily routine. Moreover, she had no difficulty discussing incidents that had occurred some 20 yrs earlier in terms that her husband corroborated. She easily recalled the plots of fairy tales and TV shows. On a shortened version of Butters' Famous Faces Test (which excluded sports figures), she demonstrated recognition of 23/40 pictures, although she could name only 13. Further, her recognition pattern showed the normal absence of a temporal gradient across the 4 decades sampled.

Additional formal tests of remote memory, which often employ a question/answer format, were precluded because of E.D.E.'s comprehension problems.

Immediate memory. Digit span was consistent at 2, with occasional correct repetitions of 3 digits forward. Digit span was retested periodically over the next several years; in February 1985, digit span was consistent at 3, whether responses were spoken or required pointing to a digit list; her current reliable digit span remains at 3 items. Word repetition showed an input-modality dissociation. When 20 4-item lists of single syllable concrete nouns were presented aurally, none was repeated in order (as instructed); and an average of 2 words per list was reproduced with a marked recency effect. When the same items were presented visually, 4-item lists were repeated without error. Six of 20 visually-presented 5-item lists were repeated in order, with an average of 4 words reproduced correctly from each list. Repetition of aurally-presented nonwords was very poor (2/12 2-word lists).

Verbal learning. Paired-associate learning (Wechsler Memory Scale) was impaired (mean = 6/21; norms for ages 40–49 yrs: mean = 13.91, SD = 3.12). Word list learning was tested in the auditory modality with high-frequency monosyllabic noun lists of various lengths. E.D.E. was able to learn a 5-item list (in order) after 4 presentations, and a (different) 10-item list (in order) after 7 presentations. (Mean performance for normal subjects on a comparable 15-item list = 14 (SD = 1.0) after 5 trials; *see* Lezak, 1983, p. 426.) Thus verbal learning, though impaired, was clearly possible.

Visual memory. E.D.E.'s ability to reproduce simple geometric figures from memory (Wechsler Memory Scale) was excellent. Her reproduction from memory of Rey's complex figure (after 1 min of study) was scored in the 50th percentile (score = 22; *see* Lezak, 1983, p. 400). Visual tasks requiring memory for order information (and arguably requiring verbal coding) were performed less well. She obtained a score of 11 (mental age = 12.5) on the Knox Cube Test (as revised by Arthur, 1947), which requires sequential pointing to a linear block array in the order of the tester's model.

Visual-spatial functions

The 'parietal lobe' battery of the Boston Diagnostic Aphasia Examination was administered since CT scan had demonstrated involvement of the right parietal lobe. Picture drawing, clock setting, stick construction, body part and finger naming, and right/left discrimination were intact. E.D.E.'s copy of Rey's complex figure was scored as 36 (virtually perfect). Calculations were impaired except for simple addition and subtraction.

In summary, E.D.E.'s clinical profile at 1 yr postonset was consistent with descriptions that have been provided previously of patients said to have a selective disturbance of auditory-verbal short-term memory (STM). Remote memory and visual memory were relatively intact (Shallice and Warrington, 1970), repetition was better when lists were presented visually than when presented aurally (Warrington and Shallice, 1972), and span was equally impaired whether a spoken or pointing response was required (Shallice and Warrington, 1977). Her speech production was also similar to that described for many patients with STM disorder; it was fluent (without articulation disorder), syntactically well formed, with occasional phonemic paraphasias that were most apparent in tasks with constrained targets (e.g., naming). Auditory comprehension was somewhat more impaired than is typically described: although single word comprehension was well preserved, comprehension of even short sentences was very limited.

The clinical profile was also revealing of several findings relevant to the issue of crossed aphasia. First, E.D.E. demonstrated no impairment of visual/spatial or constructional abilities—capacities normally associated with lesions in the right temporoparietal region. This negative finding is consistent with the view that the neuroanatomical representation of cognitive functions can be exactly reversed in cases of unusual lateralization (R. F. Fischer *et al.*, 1991). However, E.D.E. did demonstrate some affective symptoms (acute lability and agitation) that were more typical of right hemisphere involvement (Heilman *et al.*, 1985), although these symptoms have not persisted.

Experimental tests

List repetition and single word processing

A series of studies was carried out in an attempt to uncover the basis of E.D.E.'s severe list repetition deficit. Full details of these studies were reported by Berndt and Mitchum (1990), and are summarized here.

1. E.D.E.'s list repetition span was severely reduced; she could not repeat two words reliably or alphabet letters in the correct order. List repetition was not affected by word length, a finding that has been interpreted as indicating a deficient phonological short-term store within Baddeley's (1986) model of working memory (Vallar and Baddeley, 1984*a*). The phonological similarity among the to-be-repeated items affects performance with auditory but not with visual presentation, which is also attributed to a deficit within the phonological short-term store.

2. List repetition was characterized by a consistent 1-item recency effect, which is not usually found in patients of this type (Vallar and Papagno, 1986). This recency effect was evident even when responses were scored strictly for serial order, and thus it did not result from a strategy of repeating the last items first. Several experiments performed with mouthed stimuli (requiring lip-reading), and involving the presentation of a word to be ignored at the end of the stimulus lists, supported the assertion that much of E.D.E.'s immediate repetition was dependent on a persisting activation trace of auditory information, rather than on phonetic information.

3. Phonological processing was intact for pairwise comparison and discriminations among phonemes. Monosyllabic words and nonwords can be read aloud and repeated quite well, indicating relatively good basic ability to manipulate phonemic information in a variety of ways. The presentation of longer material (lists for comparison, multisyllabic words) resulted in a marked decrement in performance. Errors in repetition of multisyllabic nonwords preserved the acoustic features of the target (stress pattern, number of syllables, vowel quality) at the expense of segmental phonetic information.

4. Semantic interpretation of single words was intact in word/picture matching with closely related distractors, and in synonym judgements. In contrast, the ability to distinguish between spoken words and nonwords was impaired such that many nonwords were accepted as words. Reaction time data suggested different types of processing for words and nonwords: E.D.E. responded to words of all types as quickly and accurately as did 2 matched controls; in contrast nonwords were processed very slowly and were the basis of errors in half of all nonword trials.

The interpretation given to these findings is that a primary deficit underlying E.D.E.'s performance was a severely reduced phonological short-term store, which existed despite preserved ability to process phonological information in tasks with minimal storage requirements. This demonstration that phonological processing and phonological storage can dissociate in cases of focal brain damage is an effective counter-argument to interpretations of short-term memory impairment that are based on the co-occurrence of apparent memory impairment with deficient ability to process phonological information (Allport, 1984; Friedrich *et al.*, 1984).

E.D.E.'s performance with single words and word lists can be interpreted as reflecting this deficient phonological short-term store, if it is assumed that she could sustain a persisting activation record (Monsell, 1984) of incoming auditory information. This auditory trace is presumably sufficient to allow immediate lexical access for known words, including access to semantic information. There was some evidence that semantic information was exploited when E.D.E. was required to repeat lists, as the imageability of the words to be repeated had a significant effect on performance. Thus results of studies of her single word repetition, comprehension and lexical decision support the notion that E.D.E. could extract considerable information from the words she heard, despite poor ability to maintain words in phonetic form. The remainder of this report will consider the extent to which the information she can extract and maintain (including syntactic information, which was not examined in the earlier study) is sufficient to allow her to comprehend and to repeat sentences.

Sentence comprehension

E.D.E.'s sentence comprehension deficit was the residual clinical feature that caused her the most concern. She was often passionate in her insistence that people 'talk slowly' and 'keep it simple'. The low scores obtained on the first testing of the BDAE (1 yr postonset) have improved somewhat in the 2-yr period since that time, but serious residual problems have remained. The Test for Reception of Grammar, TROG (Bishop, 1982) was administered to E.D.E. in June 1984. This test includes 68 sentences to be matched to 1 of 4 coloured line drawings. The test is stratified from very easy 4-word items requiring only comprehension of lexical meanings to 10-word sentences requiring processing of multiclausal structures. Many of the items are semantically reversible and require interpretation of word order as well as of other syntactic cues. E.D.E. obtained an overall score of 72% correct on the 68 sentences. Performance on items requiring interpretation of word order and other syntactic cues (subtests H and K through to T) was considerably worse at 59% correct. This level of performance may not differ from chance: although 4 pictures are available on each trial, yielding a probability of correct performance through guessing of 0.25, only 1 distractor picture per trial tests comprehension of syntactic cues. The other distractors can be rejected on lexical grounds alone. Thus, a patient with relatively intact lexical knowledge could guess to decide between the 2 syntactically-related pictures and be correct with a probability of 0.50. Structural complexity, correlated in this task with length, was an important determinant of performance. On 12 sentences longer than 7 words, including 8 with relative clauses, E.D.E. was correct on only 38% of trials. Thus it appears that E.D.E.'s comprehension of sentence length materials is indeed poor.

Experiment 1 (active, passive and dative sentences). A sentence-picture matching task, containing only 2 picture choices, was administered in an attempt to uncover the aspects of sentence meaning that E.D.E. was failing to interpret. Nonreversible active and passive sentences (e.g., 'The woman is painting the wall') can be interpreted correctly on the basis of lexical meaning alone. Distractors probed the meanings of each of the three major lexical items in sentences (subject noun, verb and object noun). Reversible sentences require interpretation of word order and verb morphology for comprehension (e.g., 'The man is pushing the woman'), and distractors picture a reversal of logical roles (woman pushing man). Dative constructions, all of which were semantically reversible, were of three types, requiring different lengths. Active voice datives (e.g., 'The woman is selling a book to the man') and passive voice datives ('The woman was sold a book by the man') were paired with the correct picture and a distractor showing a reversal of logical roles. Truncated passivized datives with indirect object fronted (e.g., 'The book was sold to the man'; 'The book was sold by the man') were shorter in length but at least as complex as the other datives. These sentences were randomly combined and presented in two test sessions.

Results. Table 1 shows that the factor of semantic reversibility was a strong predictor of E.D.E.'s performance. With sentences up to 9 words in length, she performed well if interpretation could be based on lexical meaning. In contrast, performance with semantically reversible sentences was poor even when sentences were as short as 7 words in length. Relatively good performance with active reversibles must be interpreted cautiously, since performance on passives was worse than would be expected by chance. This suggests that E.D.E. may have been using a strategy of identifying the first noun heard as the actor, rather than on full comprehension of sentence meaning.

TABLE 1 PROPORTION CORRECT SENTENCE/PICTURE MATCHING

Type	*No.*	*Length (words)*	*Proportion correct*
Nonreversible			
Active and passive	24	7, 8	0 92
Longer active, passive	12	8, 9	0 83
Reversible			
Active	8	6	0.88
Passive	8	7	0 25
Dative			
Active	6	9	0.66
Passive	6	9	0.00
Truncated	20	7	0.50

Experiment 2 (sentence-picture matching with locative relations). Sentences with locative prepositions allow investigation of a patient's difficulties with word order in a context where other aspects of syntactic complexity (e.g., verb morphology) are kept to a minimum, and where the factor of reversibility can be probed using different distractor items with the same sentences. Simple sentences with a locative preposition expressed a relationship between 2 objects (e.g., 'The barrel is above the box'). In the 'preposition distractor' condition, the distractor picture showed an incorrect locative relationship between the 2 objects (barrel behind box). In the 'reversal distractor' condition, the locative relation is reversed (box above barrel). These items were increased in length by the addition of a single adjective to modify 1 of the 2 nouns (e.g., 'The blue barrel is above the box'). This additional word was not relevant to performance of the task, as both the target picture and the distractor showed, for example, a blue barrel. The lengthier version of this task was administered twice, 1 yr apart.

Results. Data from the two administrations of the longer task were combined since the same pattern of results was obtained. As can be seen in Table 2, the short version of this task was performed with no errors, even with the reversal distractor. The addition of 1 word to the sentences, however, undermined performance with the reversal distractor. When the adjective was added to the first noun of the sentence, performance fell to chance levels.

TABLE 2. PROPORTION CORRECT SENTENCE-PICTURE MATCHING WITH LOCATIVE PREPOSITIONS

Length (words)	*Distractor*	*No.*	*Proportion correct*
6	Preposition	16	1.00
6	Reversal	16	1.00
7	Preposition	32	1.00
7	Reversal	32	0.72
	[Early modifier	16	0.56
	Late modifier	16	0.88]

Experiment 3 (auditory judgements of grammaticality). Forty-eight tape-recorded sentences were presented to E.D.E., and she was asked to judge whether or not they were grammatically correct. Items were selected from a total of 451 sentences constructed and recorded by Linebarger *et al.* (1983). Violations included 4 examples of 6 of the violation types used by those authors. Tapes were made by rerecoding the original stimuli developed by Linebarger *et al.*, using recording procedures that effectively remove any prosodic cues to syntactic ill-formedness that might exist in the aberrant sentences (Berndt *et al.*, 1988). Each item was presented to E.D.E. twice. Grammatical and ungrammatical versions of the same item were divided randomly into two blocks, which were administered approximately 1 month apart.

Results. E.D.E. correctly judged the grammaticality of 47/48 items.

In summary, tests of E.D.E.'s sentence comprehension indicated a rather clear interaction between syntactic factors and sentence length. Her ability to understand semantically-reversible sentences—which required interpretation of the logical relationship between the verb form or preposition and the sentence nouns—was intact in short sentences (6 or fewer words). However, this ability deteriorated markedly if a single word was added. It is not clear why the additional word occurring early in the sentence was more disruptive of performance than was a word occurring later, since in either case comprehension could be based solely on the fragment of the sentence without the additional modifier (e.g., 'the box is over . . .' or '. . . over the barrel'). It is possible that the early modifier interfered with the immediate on-line construction of a syntactic representation as the sentence was heard, and that E.D.E. failed to recover from this initial disruption even though sufficient information was available later in the sentence. This analysis garners some support from her good performance of the grammaticality judgement task, which suggested relatively normal *immediate* construction of a syntactic representation.

The following experiments were carried out in an attempt to investigate the influence of sentence structure and content on E.D.E.'s sentence repetition.

Sentence repetition

Experiment 4 (repetition of active and passive sentences). Sixteen active and passive sentences from the same set as were used to test comprehension were given to E.D.E. for repetition. They were presented once, in random order. Active voice sentences were 6 words in length; passive voice were 7 words long.

Results. E.D.E. repeated verbatim 9/16 sentences, 5 active and 4 passive. When she failed to repeat, she usually (6/7) repeated the subject noun phrase and the end of the sentences and omitted the middle. There was no tendency to paraphrase; she most often simply omitted a word, or substituted the word 'something' and proceeded to the remainder.

Repetition of ungrammatical sentences. Eight ungrammatical sentences and their grammatical partners were selected from the Linebarger *et al.* materials that were used in Experiment 3. Items were 6–8 words in length, with a variety of constructions. Grammatical sentences were presented for repetition approximately 1 month after repetition of ungrammatical sentences.

Results. E.D.E. repeated 2/8 sentences correctly in each of the conditions. Two errors in the ungrammatical condition involved correction of the sentence, but other errors occurred in the same items. The character of repetition errors was generally the same regardless of the grammaticality of the stimulus: again, she failed to repeat the middle of the sentence. Despite comparable performance on these items, however, presentation of the ungrammatical sentences repeatedly elicited the comment that 'something's wrong', or 'that's not right'.

Experiment 5 (repetition of sentences with a variety of constructions). Six types of sentence, 6–9 words in length, were constructed in which verbs occurred either early, midway or late in the sentence. For each verb position, 2 different sentence types were employed that manipulated the relative morphological complexity of the verb. Twelve different tokens of each sentence type were combined to form 1 of 6 blocks for administration. Two blocks were given in each of 3 sessions, several months apart.

Results. E.D.E. repeated verbatim 17% of the 72 sentences, with no consistent advantage or disadvantage for different sentence types. Serial position curves were generated for all of the 7-word sentences (a total of 47 sentences across the 6 types). Fig. 4 presents the mean proportion of words recalled from each of the 7 word positions across all sentence types. Regardless of verb position or syntactic complexity, E.D.E. tended to reproduce the first and last words of the sentence and to omit the midportions. Again, there was no tendency for paraphrase that would indicate meaning-driven repetition performance.

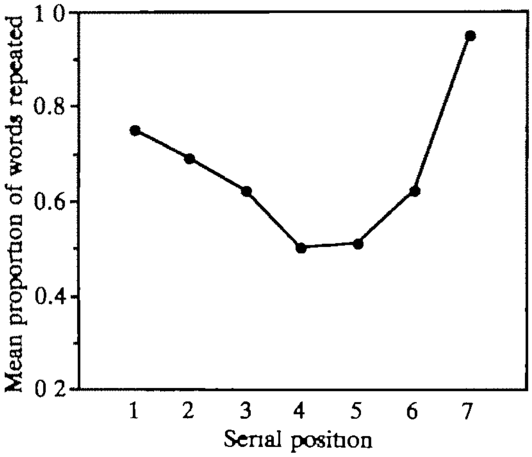

FIG. 4. Mean proportion of words recalled from each of 7 serial positions, collapsed across 6 sentence types

Experiment 6. This experiment was designed to explore further the apparent lack of syntactic effects on repetition as well as to investigate the importance of semantic coherence as a predictor of performance.

Stimulus sentences were selected from materials developed by Saffran and Martin (unpublished). Five conditions were included: a base sentence, with normal syntactic and semantic structure; 2 semantic violations (an anomalous verb substitution and an exchange of subject and object nouns in the base sentence); a semantically unconstrained version in which content words were randomly substituted from the base sentences while syntactic structure was maintained; and a scrambled version produced by randomly ordering the words of the base sentences. Six 7-word sentences of each type were combined and randomized for administration. These were presented to E.D.E. twice, 1 month apart.

Results. E.D.E. did not repeat any of the sentences perfectly. Table 3 presents the mean number of words repeated for each sentence type, and indicates that there was little effect of the verb substitution and reversal of word order. In fact, there was only a slight decrement in performance when sentences were semantically unconstrained (χ^2 (df=1) = 0.39). However, the scrambled sentences, which were devoid of syntactic structure but maintained some semantic coherence among content words, were repeated significantly more poorly than were the base sentences (χ^2 (df=1) = 12.66, $P < 0.001$). There was no tendency to repeat content words and to delete functors, even in the scrambled condition (proportion substantives/total words = 0.61 for base and 0.59 for scrambled sentences).

TABLE 3 REPETITION OF WELL-FORMED AND ANOMALOUS 7-WORD SENTENCES (EXPERIMENT 6)

*Type**	*Example*	*Mean no. words repeated*
Base	Any nurse can cut off the bandage	4.3
Semantic violation (verb substitution)	Any nurse can shut off the bandage	4.1
Semantic violation (reversal)	Any bandage can cut off the nurse	3 8
Semantically unconstrained	The stick can shut around her sweater	3.0
Scrambled	Can off nurse any the cut bandage	2 3

* n = 12 sentences/types.

The following experiment was designed to investigate whether E.D.E. could be encouraged to use syntactic and semantic information when repeating by being allowed to determine the size of the chunk to be repeated.

Experiment 7 (text segmentation with repetition). This task is a modification of one used by Wingfield and Butterworth (1984) to assess the contribution of on-line syntactic analysis to retrieval from working memory. A semantically-coherent 83-word story, containing 7 sentences with a variety of syntactic structures, was taken from memory tests discussed by Lezak (1983). The passages were tape-recorded by a female speaker with normal narrative intonation and rate. E.D.E. and 2 matched control subjects were asked to listen to the passage and to stop the recorder at whatever point yielded a segment they felt comfortable repeating.

Results. The normal control subjects performed very similarly to those tested by Wingfield and Butterworth. Mean segment length was 13.83 words, and 7/12 segments occurred at sentence boundaries; 87% of words were recalled correctly. E.D.E. produced 23 segments, with a mean length of 3.56 words, and repeated 65% of the words correctly. Although she successfully segmented the text at the termination of all sentences, intrasentential breaks frequently violated constituent structures (0.32 of segments). Moreover, her ability to repeat the segment correctly was not predicted by how well-formed was the syntactic 'unit' that she had created.

In summary, the results of these tests of E.D.E.'s sentence repetition indicated some reliance on syntactic and/or prosodic information, as repetition deteriorated significantly relative to a baseline condition when the words of sentences were scrambled. However, performance was not affected by minor syntactic anomalies, even though it was clear that these were detected. In addition, structural complexity and verb position had little effect on repetition. Even when E.D.E. was encouraged to use syntactic structure to define her own 'optimal' unit for repetition, she failed to exploit this information effectively. Semantic coherence had a minimal effect on repetition performance, and the quality of her repetition responses—devoid of paraphrase—does not appear to reflect semantic information.

DISCUSSION

The pattern of results reported here for E.D.E. is in many ways similar to findings that have been reported for other patients with putative short-term memory impairment. In addition to the general neuropsychological profile, experimental results that are consistent with previous reports are the following. (1) The pattern of list repetition is one in which written words are repeated better than spoken words and word length does not affect recall; (2) repetition of phonemically-related items is poorer than of unrelated items only when presentation is auditory; (3) single spoken words are comprehended well, but sentence comprehension is impaired; and (4) sentences are produced fluently and are syntactically well-formed, with no overt indication of articulatory disorder, grammatical morpheme omission or structural abnormalities. E.D.E.'s symptoms are thus very similar to those reported for other patients with short-term memory deficit, and the lesion giving rise to these symptoms in the right hemisphere is homologous to the superior temporal, inferior parietal locus of the lesions reported for similar patients (Warrington *et al.*, 1971; Saffran and Marin, 1975; Caramazza *et al.*, 1981; Friedrich *et al.*, 1984). E.D.E. might be interpreted, therefore, as presenting a 'mirror image' crossed aphasia (Alexander *et al.*, 1989). However, there are other aspects of her performance that differ in potentially important ways from the performance of these other patients.

First, although the evidence is strong that E.D.E. demonstrates a deficient phonological short-term store, her ability to discriminate between similar phonemes is intact (Berndt and Mitchum, 1990). Discrimination of speech sounds has been found to be deficient in many of the patients with memory-deficit in whom this function has been investigated (Allport, 1984; Friedrich *et al.*, 1984; Campbell and Butterworth, 1985; *but see* Vallar and Baddeley, 1984*b*). The co-occurrence of a phoneme discrimination deficit with short-term memory limitation supports the contention that the memory deficit is functionally related to, or is even secondary to, an inability to analyse speech sounds for subsequent storage (*see* Allport, 1984, for an argument along these lines). Thus the clear dissociation of memory limitation from phonemic analysis skills in E.D.E. constitutes evidence that short-term memory can be affected by focal brain damage independently of other aspects of the speech processing system.

Another aspect of E.D.E.'s list repetition performance that differs from the patterns exhibited by similar patients may be related to her intact phoneme discrimination skills. She produced a recency effect in immediate serial recall; other patients' recall curves have shown primary effects only (Vallar and Papagno, 1986). We have interpreted this recency effect as reflecting E.D.E.'s ability to store information in the form of a nonphonological, auditory code (Berndt and Mitchum, 1990). The apparent sparing of memory for auditory events in E.D.E., and impairment of such memory in other patients, is likely to be linked to our patient's intact ability to process speech sounds up to the point at which storage is required. For other patients with short-term memory impairment, poor auditory memory and poor phonemic processing may be functionally linked.

Thus in comparison with other patients with reported short-term memory involvement, E.D.E. shows particularly good processing of the incoming speech signal. Nevertheless, she is incapable of maintaining phonemically coded information beyond a few items. Her sentence processing capacities also appear to differ from those of other similar patients in that it is more severely impaired. Although a wide range of comprehension deficits has been described for similar patients, many have shown impairment only when sentences are syntactically quite complex (e.g., Vallar and Baddeley, 1984*b*; Martin, 1987) or require integration of information from a variety of sources (e.g., McCarthy and Warrington, 1987*b*). Moreover, E.D.E.'s comprehension disorder has been shown to reflect an interaction of syntactic factors with sentence length, such that sentences requiring interpretation of word order are poorly understood only when they exceed a critical length. Thus it is difficult to argue that E.D.E. suffers from an inability to interpret syntactic cues per se. Some impairment in the ability to retain information during sentence comprehension appears to be implicated by this interaction of structural factors with length.

Recent attempts to explain the link between short-term memory impairment and comprehension deficit have argued that the memory system that supports list repetition, and the system involved in processing sentences for comprehension, must be different (Caplan *et al.*, 1986; McCarthy and Warrington, 1987*b*; Martin, 1987). We have attempted to assess directly the extent to which E.D.E. maintains *sentences* using a variety of sentence repetition procedures, and all indications are that E.D.E. relies on the same information when she repeats sentences as she does when she repeats lists. That is, sentence repetition is not affected by semantic well-formedness and is not characterized by meaning-based paraphrase, unlike results reported for similar patients (e.g., Saffran and Marin, 1975; Caramazza *et al.*, 1981). In fact, there is little evidence in the results reported that E.D.E. generates a semantic representation for the sentences that she repeats. Instead, she appears to repeat sentences on the basis of the same (inadequate) phonemic and auditory codes that support repetition of lists.

It is also clear, however, that E.D.E. does compute a syntactic representation for sentences. This is evidenced by her good performance of the grammaticality judgement task, and by her significantly poorer repetition of scrambled than of well-formed sentences. Nonetheless, she was not able to exploit syntactic structure to 'chunk' to-be-repeated information into constituents. There is thus considerable evidence that, despite intact structure-building operations, E.D.E. is deficient in the ability to use the syntactic representation she can compute to construct a semantic representation, or to guide sentence repetition.

Saffran (1990) has presented a detailed consideration of several possible functional explanations of sentence comprehension disorder among patients whose short-term memory impairment is attributable to inadequate phonological storage. Saffran has proposed that the functional locus of the comprehension deficit does not involve an inability to construct a syntactic representation, i.e., to parse the spoken input into a representation that expresses abstract constituents such as subject, verb and object. E.D.E.'s good performance of the grammaticality judgement task supports this assertion. Instead, Saffran argues that the patient fails to comprehend the sentence because of an inability to retain the lexical correspondents—held in veridical, phonological form—while they are assigned to these abstract syntactic categories. Patients show a variety of comprehension patterns, it is argued, because they can adopt a variety of strategies for coping with the difficulty they have maintaining the phonological forms that must be mapped onto the output of this syntactic analysis. Many patients, including E.D.E., appear to adopt a strategy of assigning the thematic role of agent to the first possible noun phrase that is encountered, resulting in correct interpretation of active voice sentences. Otherwise, patients will rely on other information that may be available, such as partial semantic analysis. Thus patients who paraphrase when repeating, or who are strongly affected by the semantic well-formedness of the to-be-repeated material, are presumably relying on an ability to generate some aspects of the meaning of a sentence which they can maintain even after the phonological form is lost.

E.D.E. behaves quite differently. Despite good comprehension of meaning at the single word level, and good discrimination between targets and *lexical* distractors in sentence comprehension, she shows very little evidence of meaning-driven sentence repetition responses. The picture that emerges is somewhat paradoxical: despite *better* ability to analyse auditory/phonological information than is shown by other, similar patients, E.D.E. demonstrates *poorer* ability to gain access to meaning in sentences. One possible explanation, suggested by Saffran's analysis, is that E.D.E.'s reliance on auditory/phonological information in attempting to repeat is a strategic response to her poor maintenance of phonetic information. Unlike other patients, E.D.E. may be slow to abandon reliance on the sounds of the incoming stimulus sentence, since her initial processing of those sounds may be largely unimpaired. Patients who are experiencing very early processing difficulties may be more likely to focus all attempts on the immediate extraction of some meaning from the speech stream.

From the viewpoint of the neuroanatomical considerations raised in regard to crossed aphasia, these results suggest a point of departure for future studies. One intriguing alternative to the analysis offered above is that the normal organization of the language system in right-handed individuals provides for strict left hemisphere representation of auditory/phonological processing and storage, with some involvement of the right hemisphere in lexical/semantic analysis. Thus the cases of short-term memory impairment with left temporoparietal damage may show deficient phonological processing and storage, with preserved semantic access in the face of these deficits, as a consequence of the representation of those functions and their sites of lesion. For E.D.E., whose pattern of cognitive representation is presumably abnormal, there appears to be an unusual dissociation of functions such that the perception of auditory/phonetic information is separated from its storage, while access to semantic information from phonemic forms in connected speech is impaired. It is possible to speculate that some initial processing of auditory/phonetic information is carried out in E.D.E.'s intact left hemisphere, while language functions responsible for phonetic storage and lexical/semantic assignment to sentence constituents are lateralized to the right hemisphere. More detailed analysis of such dissociations in other patients with unusual lateralization of function could contribute important information to our understanding of the normal representation of cognitive function.

ACKNOWLEDGEMENTS

The authors are grateful to E.D.E. and to her husband, J.E., for their willingness to participate in this study, and to Eleanor Saffran for making available the stimulus materials used in Experiments 3 and 6.

Special thanks to Maryne C. Glowacki for preparing the manuscript and the tables. Preliminary data from this study were presented at the Venice meeting on Cognitive Neuropsychology (March 1985), as part of a Symposium on Working Memory Disorders at the Academy of Aphasia (October 1985), and at a conference on Neuropsychological Investigations of Short-term Memory, Lake Como, Italy (October 1987). This study was supported by NIH grants NS21054 and NS16332 to the University of Maryland Medical School.

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(*Received November 3, 1989. Revised January 19, 1990. Accepted March 6, 1990*)

Brain (1991), **114**, 281–294

MAINTENANCE OF MYELINATED FIBRE g RATIO IN ACUTE EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS

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SUMMARY

The spectra of myelin sheath thickness and g ratio (axon diameter/fibre diameter) of guinea pig optic nerves for 8 animals with acute experimental allergic encephalomyelitis (EAE) were compared with those for 6 normal animals. The mean myelin sheath thickness of 0.12 μm for the animals with EAE was significantly lower than the value of 0.16 μm for the normal animals. Since fibre diameter comprises axon diameter plus the thickness of its surrounding myelin sheath, a reduction in mean fibre diameter from 1.52 μm in normals to 1.20 μm in EAE was expected, but it was surprising to find that a mean g ratio of 0.85 obtained for normal nerves was not substantially different from a value of 0.86 for demyelinated optic nerves. A decrease in the mean axon diameter of 1.24 μm for normal animals to 0.94 μm for those with EAE tended to offset the decrease in mean myelin sheath thickness and contributed to the relative stability of the g ratio with acute demyelination. Our results showing reduction in axonal calibre and myelin sheath thickness may offer an explanation for apparent discrepancies between electrophysiological delays in conduction characteristics of experimental and, if not the result of a maturational effect on myelination, human primary demyelinating disorders associated with the scant histopathological demyelination of initial attacks of EAE and the visually asymptomatic patients with multiple sclerosis.

INTRODUCTION

Experimental allergic encephalomyelitis (EAE) is an immune-mediated demyelinating disorder of the central nervous system (CNS) induced by sensitization to central myelin (Waksman and Adams, 1962; Wisniewski and Madrid, 1983). Limb paralysis, gait ataxia and visual loss characteristic of EAE resemble the deficits observed in patients with acute disseminated encephalomyelitis and multiple sclerosis (MS) (Alvord, 1970; Lidsky *et al.*, 1980; Lassmann, 1983*a*, *b*; Raine, 1985; Shaw *et al.*, 1988). Sensitization of adult strain 13 guinea pigs produces an acute form of EAE (Alvord, 1970; Rao *et al.*, 1977), whereas sensitization of juvenile animals results in a relapsing and remitting form of the disease (Wisniewski and Madrid, 1983). Histopathological examination of the optic nerves and other myelinated CNS tracts of EAE animals has disclosed perivascular cuffing, interstitial inflammation and demyelination (Adams, 1959; Rao, 1981; Wisniewski and Madrid, 1983; Shaw *et al.*, 1988).

The anatomy of the guinea pig optic nerve (Rao, 1981; Guy *et al.*, 1989*c*) is similar to that of the primate (Potts *et al.*, 1972*a*, *b*; Sanchez *et al.*, 1986) and other species

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in which myelination commences posterior to the lamina scleralis. Impulse velocity of myelinated fibres is increased by saltatory conduction. Leakage of current is reduced by the myelin sheath functioning as an insulator (Huxley and Stämpfli, 1949). Myelin sheath thickness increases concomitantly with axon diameter, maintaining a relatively stable g ratio (axon diameter/fibre diameter (Rushton, 1951; Bishop *et al.*, 1971).

A g ratio of between 0.60 and 0.75 is considered to be optimal for conduction of the action current (Goldman and Albus, 1968; Jack *et al.*, 1975). While conduction on remyelinated nerves may be maintained as the distance between nodes of Ranvier decreases (Bostock and Sears, 1976, 1978), the effect of EAE on alterations in the g ratio has not been systematically examined in the optic nerve. An increase in the g ratio implies a relative reduction in the thickness of the myelin sheath, as fibre diameter approaches that of axon diameter (Beuche and Friede, 1985).

There has been a lack of definitive clinicopathological correlation of histopathological lesions with clinically apparent neurological deficits (Mackay and Hirano, 1967; Ghatak *et al.*, 1974; Waxman, 1981) and alterations in conduction velocity (Halliday *et al.*, 1972; Chiappa, 1980; Bilbool *et al.*, 1983; Hume and Waxman, 1988) both for human disorders and animal models of primary demyelination. While remyelination of the CNS occurs in MS, clinical remissions may be seen before remyelination (Davis, 1970; McDonald, 1974; Prineas and Connell, 1979; Smith *et al.*, 1982; Kaji *et al.*, 1988; Raine and Scheinberg, 1988).

In order to elucidate further the observed discrepancies implied by the increases in the latency of visual evoked potentials in patients with MS and no apparent visual symptoms (Halliday *et al.*, 1972; Hume and Waxman, 1988) and the scant histopathological demyelination of the optic nerve with initial attacks of EAE (Lidsky *et al.*, 1980; Hayreh *et al.*, 1981; Bilbool *et al.*, 1983), we performed a detailed morphometric analysis of myelin sheath thickness, fibre calibre, axon calibre and g ratio of optic nerves from guinea pigs with EAE as compared with normal controls without EAE.

MATERIAL AND METHODS

Production of EAE

Fourteen female strain 13 guinea pigs aged 1 month weighing 240–300 g were purchased from Crest Caviary (Raymond, CA): 8 animals were randomly chosen to receive a subdermal injection into the nuchal area with 0.5 ml of an emulsion of spinal cord in complete Freund's adjuvant (Difco Laboratories, Detroit, MI) and 6 unsensitized animals served as the control group. All sensitized animals developed limb paralysis and were killed by intracardiac injection with 3 ml of pentobarbital 2 wks after antigenic sensitization. The weight of the individual animals at the termination of the experiment is given in Table 1.

Histological preparation

The right globes and attached optic nerve were immediately dissected out and fixed overnight in cold 1% glutaraldehyde-4% paraformaldehyde buffered with 0.1 M sodium cacodylate HCl (pH 7.2). The optic nerves were incized approximately 3.0 mm from the scleral insertion, rinsed in 0.1 M cacodylate buffer, postfixed overnight in 1% buffered osmium tetroxide, dehydrated through an ethanol series to propylene oxide, and then infiltrated and embedded in epoxy resin.

Ultrathin (80 nm) cross-sections of the optic nerve, sectioned 3.0 mm from the scleral insertion, were poststained with uranyl acetate followed by lead citrate for examination by transmission electron microscopy. In order to sample representative areas of the normal optic nerve, micrographs were taken from central and peripheral zones depicted by the confines of the asterisks in fig. 1.

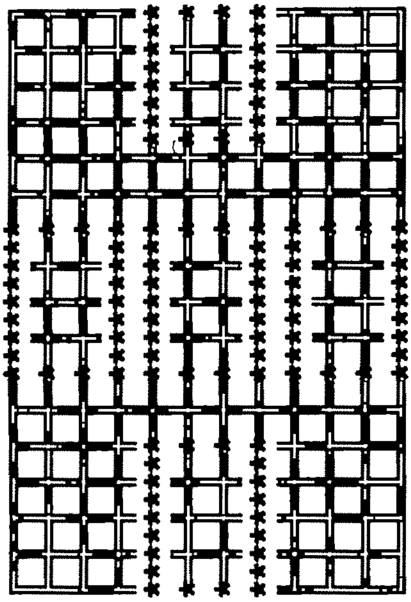

FIG. 1 Diagram of an EM grid holding the specimen shows the central and peripheral zones of the photographed optic nerve, cross-sections are surrounded by asterisks

Electron micrographs were taken with meticulous reference to the x and y grid coordinates, ensuring that duplication of photographs did not occur over the same area. The supporting grids prohibited photographing or viewing that part of the specimen obscured by the grid bars. The optic nerves were photographed on 3 25 × 4.00 inch negatives at a magnification of ×2500 and printed to a final magnification of ×7000. Magnification was calibrated with a line grating. After a fibre distribution was obtained for normal animals, micrographs representative of demyelination were selected from the 4 peripheral quadrants and the centre of the optic nerve cross-sections of EAE animals.

Morphometric analysis

In order to obtain the spectra of myelin sheath thickness and the g ratio of the normal guinea pig optic nerve, the myelin sheath (outer diameter) and the axolemma (inner diameter) of 68 785 optic nerve fibres of the 6 normal animals, and 17 337 fibres of the 8 guinea pigs with EAE, were traced manually with the electronic pen of an Apple graphics tablet. Using an Apple IIe microcomputer, the data were stored on floppy diskettes.

The magnification and graphics tablet resolution allowed a final linear resolution of 138 points per μm. Repetitive measurements of identical fibres were found to vary less than 0.33%. Since fibres were not perfectly circular, axon diameter, fibre diameter and myelin sheath thickness were obtained from the area measurements. Williams and Chalupa (1983) have shown this method of calculation to vary by 8% with a 30% oblique sectioning of the nerve. Longitudinally sectioned fibres and those with fixation artefacts preventing accurate tracings were excluded from analysis. Corrections for tissue shrinkage due to fixation and sectioning were not attempted. Since no difference in the fibre spectra was found in the central versus peripheral sectors of normal guinea pig optic nerves, fibres of normal optic nerves were pooled for comparisons with those of demyelinated nerves

Statistical analysis of the data was performed using the S-Plus statistical language on a Sun 386i workstation. Random coefficients regression was used to adjust for the thousands of measurements made on each animal. Average values and their standard errors were calculated for each animal. These estimate the mean for each animal, which had thousands of measurements. These estimates were then used in a weighted analysis of variance to estimate the experimental group effects. For each animal, we performed a regression

$$\text{axon} = \beta_0 + \beta_1 * \text{fibre} + \epsilon,$$

where ϵ is taken to be a random error term. This regression was used to eliminate any measurement pair (outer and inner diameters) with a residual error greater than 5 SD from 0. These outliers presumably arose from errors in the measurement process, e.g., lifting the electronic pen while tracing the axon or fibre. While there was variation in the number of deleted measurement pairs by animal, in no case was the number of deleted measurements greater than 3% of the total (Table 1). The validity of this linear model was assessed using residual analysis (Belsley *et al.*, 1980).

Using the data sets obtained, the average myelin sheath thickness, average fibre diameter, average axon diameter and average g ratio as measured by β_1 from the regression equation were calculated for each animal. These estimates were then combined in a weighted analysis of variance using the SE estimates to weight these observations.

TABLE 1 SUMMARY OF ANIMAL WEIGHT (g) AND ANALYSED FIBRES

Animal	*Wt (g)*	*No. of fibres traced*	*No. of fibres in analysis*	*No. of fibres eliminated*	*Total (%)*
c1	470	25503	24834	669	2 6
c2	695	30124	29822	302	1 0
c3	415	10776	10574	202	1 9
c4	493	1485	1481	4	0.3
c5	415	1071	1066	5	0 5
c6	598	1018	1008	10	1 0
a1	566	1740	1724	16	0.9
a2	601	3789	3712	77	2.0
a3	486	1098	1089	9	0.8
a4	565	3155	3113	42	1.3
a5	560	2507	2489	18	0.7
a6	540	1338	1318	20	1 5
a7	475	1678	1664	14	0.8
a8	530	2238	2228	10	0 4

c1–c6 = normal control animals; a1–a8 = animals with EAE, wt = weight.

RESULTS

Myelin sheath thickness

Fig. 2 shows a representative micrograph from a guinea pig with EAE. Small naked axons and thin sheaths of myelin surrounding larger fibres are clearly seen. Mean myelin sheath thickness was 0.16 μm for all fibres obtained from normal animals, but decreased to 0.12 μm in those with EAE. This difference was statistically significant ($P = 0.001$). A summary of average myelin sheath thickness is shown in Table 2. Average myelin sheath thickness for each animal in the normal group ranged from 0.11 μm to 0.18 μm, whereas for each animal with acute EAE this value was shifted towards thinner sheaths of myelin that ranged from 0.09 μm to 0.16 μm. It is evident that the average myelin sheath thickness of 4 of the 8 animals (a1, a2, a6, a7) with acute EAE had values that were less than or equal to the smallest value obtained for any animal (c1) in the normal group (fig. 3). Moreover, the largest average myelin sheath thickness obtained for guinea pigs with acute EAE was still less than the values obtained for 3 of the 6 normal animals.

Thin sheaths of myelin less than 0.10 μm in thickness surrounded 9.9% of all the fibres in the normal group, whereas 25% of the fibres obtained from animals with EAE

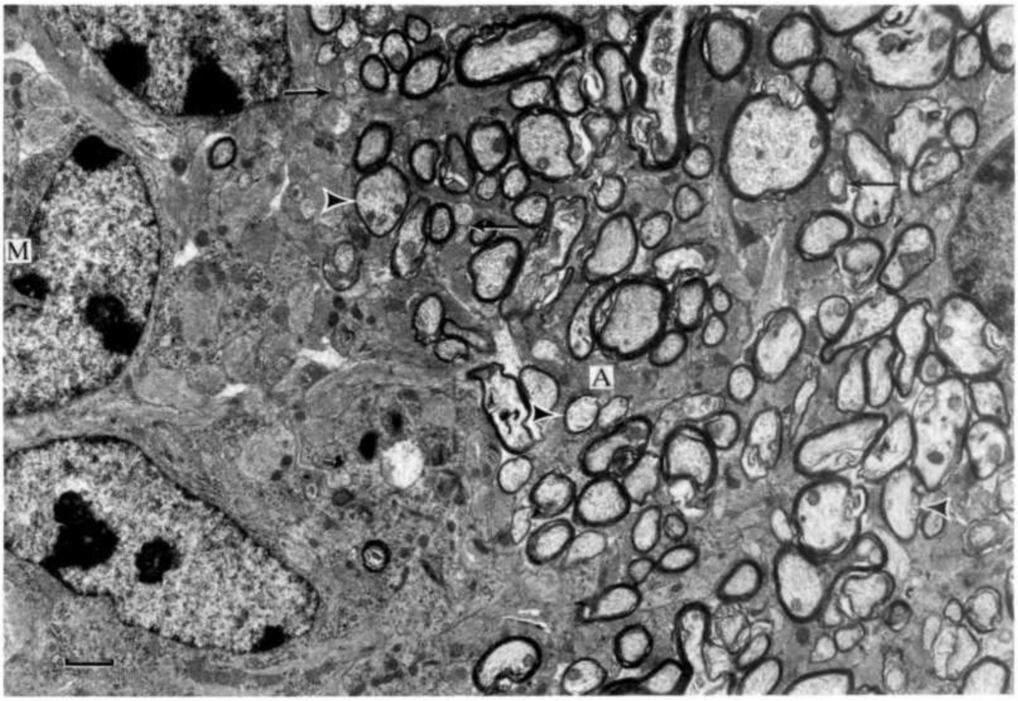

FIG. 2. A representative optic nerve micrograph of an animal with EAE illustrates small naked axons (arrows) and thin sheaths of myelin surrounding larger axons (arrowheads). Mononuclear cells (M) and astroglial processes (A) are also apparent. Bar = 1 μm.

TABLE 2. MYELIN SHEATH THICKNESS

Animal	*Average (μm)*	*SD*
Normal		
c1	0.113	0.091
c2	0.131	0.054
c3	0.145	0.061
c4	0.166	0.048
c5	0.172	0.050
c6	0.179	0.050
EAE		
a1	0.087	0.055
a2	0.092	0.046
a3	0.162	0.052
a4	0.131	0.052
a5	0.142	0.044
a6	0.103	0.050
a7	0.111	0.048
a8	0.158	0.052

had sheaths of myelin of this calibre. While completely naked axons represented 3% of the fibres in animals with EAE, unmyelinated axons were not observed in the normal guinea pig optic nerve except for fibres sectioned through nodes of Ranvier.

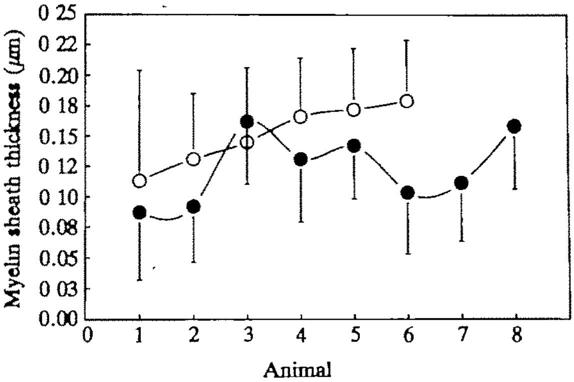

FIG 3. Average myelin sheath thickness and SD bars for animals with acute EAE (filled circles) and normal controls (open circles)

Fibre size distribution

As anticipated, there was a shift of the entire EAE function to the left towards smaller diameter fibres in comparison with the normals (fig. 4). Since fibre calibre comprises axon diameter plus the thickness of its surrounding myelin sheath, a reduction in mean fibre diameter was predictable. The mean fibre diameter of 1.20 μm for EAE animals was significantly lower than the mean fibre diameter of 1.52 μm observed for normal animals ($P = 0.002$). Average fibre diameters for normal animals ranged from 1.24 μm to 1.60 μm, while those with acute EAE had a range of 1.16 μm to 1.73 μm (Table 3). Six of 8 guinea pigs with EAE had average fibre diameters that were less than the animal with the lowest average value for the normal group. We noted that 1 of our animals with EAE (a3) had the largest average diameter observed in both groups, although as evidenced by the higher SD and lower sample size for that animal, that estimate was less precise than the other estimates.

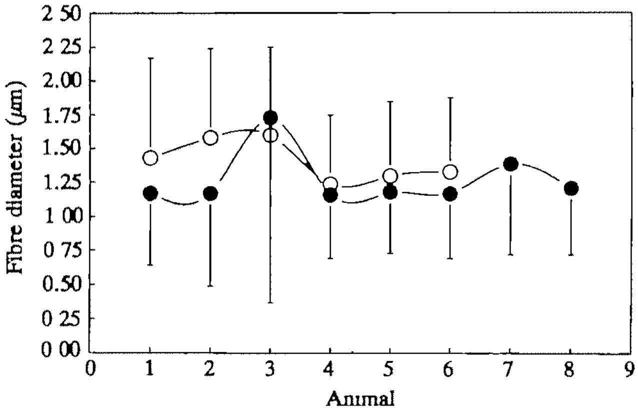

FIG. 4 Average fibre diameter and SD bars for guinea pigs with acute EAE (filled circles) and normal controls (open circles)

TABLE 3 FIBRE DIAMETER

Animal	*Average (µm)*	*SD*
Normal		
c1	1.43	0.74
c2	1.58	0.66
c3	1.60	0.65
c4	1.24	0.51
c5	1.30	0.55
c6	1.33	0.55
EAE		
a1	1.17	0.53
a2	1.17	0.68
a3	1.73	1.36
a4	1.16	0.47
a5	1.18	0.45
a6	1.17	0.48
a7	1.39	0.67
a8	1.21	0.49

TABLE 4 g RATIO

Animal	*Average (µm)*	*SE*
Normal		
c1	0.83	0.001
c2	0.87	0.000
c3	0.85	0.001
c4	0.81	0.002
c5	0.81	0.002
c6	0.80	0.002
EAE		
a1	0.87	0.002
a2	0.89	0.001
a3	0.89	0.001
a4	0.80	0.001
a5	0.81	0.002
a6	0.84	0.002
a7	0.87	0.001
a8	0.79	0.002

g ratio

A significant rise in the g ratio (axon diameter/fibre diameter) has been anticipated with the demyelination of the optic nerve in acute EAE. It was surprising to find that the mean g ratio of 0.86 for fibres obtained from guinea pigs with EAE was not substantially different from the mean value of 0.85 obtained for the normal animals ($P > 0.70$). The average g ratio for animals in the normal group ranged from 0.65 to 0.75, while it ranged from 0.62 to 0.80 in those with EAE (Table 4). Fig. 5 shows there was substantial overlap between the groups with respect to the average g ratios. While it is impossible to prove equivalence between the average g ratios for these two groups statistically, based on our data we can be about 95% confident that the true difference in g ratios is no larger than 0.04.

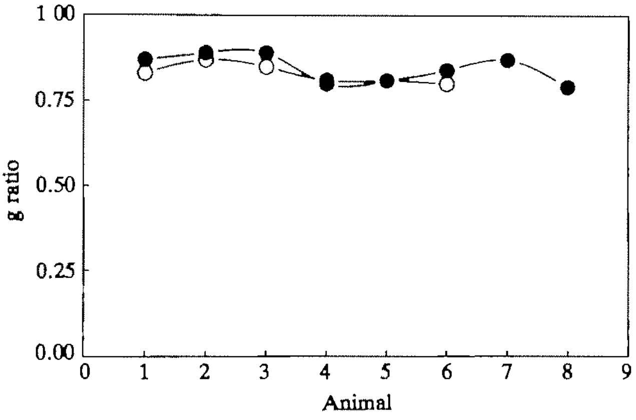

FIG 5 Average g ratio for animals with acute EAE (filled circles) and normal controls (open circles) SE bars were too small to be represented on scale

Axon size distribution

Scrutiny of the axonal spectra provided the clue to the relative stability of the mean g ratio in acute EAE. In demyelinated optic nerves, there was a shift of the axon spectrum towards smaller diameters (fig. 6). A decrease in mean axon diameter, from 1.24 μm obtained for all normal animals to 0.94 μm in the EAE group, tended to offset the decrease in myelin sheath thickness in the latter. This effect was statistically significant ($P = 0.002$) and contributed to the relative stability of the mean g ratio of EAE animals that was not substantially different from the value for normal animals. Average axon diameter for each of the normal animals ranged from 0.91 μm to 1.32 μm and from 0.89 μm to 1.44 μm for each of the guinea pigs with EAE (Table 5). Three EAE animals had average axon diameters that were less than the smallest value obtained for a normal animal. Again we note that 1 animal with EAE had the largest average axon diameter of all animals, but the precision of that measurement was low.

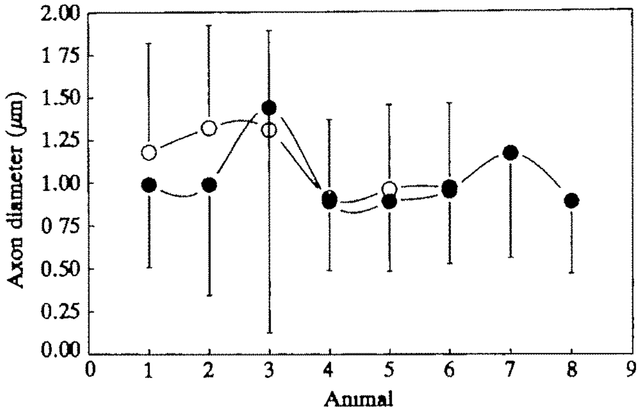

FIG 6. Average axon diameter and SD bars for guinea pigs with EAE (filled circles) and normal controls (open circles)

TABLE 5 AXON DIAMETER

Animal	*Average (μm)*	*SD*
Normal		
c1	0.18	0.64
c2	1.32	0.60
c3	1.31	0.58
c4	0.91	0.46
c5	0.96	0.49
c6	0.97	0.49
EAE		
a1	0.99	0.48
a2	0.99	0.65
a3	1.44	1.31
a4	0.89	0.40
a5	0.89	0.40
a6	0.95	0.42
a7	1.17	0.61
a8	0.89	0.42

DISCUSSION

Our morphometric study clearly shows a significant decrease of myelin sheath thickness that was observed for a majority of optic nerve fibres obtained from guinea pigs with acute EAE. Naked axons, indicative of classical demyelination, represented only a small fraction (3%) of the fibres contributed by animals with EAE. The frequency of fibres with thin sheaths of myelin, less than 0.10 μm in the group of guinea pigs with acute EAE (25%), was more than double that of fibres of the normal group with a comparable calibre (9.9%).

The cause that reduced myelin thickness in acute EAE is unknown. Fibres with thin sheaths of myelin may represent regrowth of myelin, as reported for MS (Prineas *et al.*, 1969; Prineas and Connell, 1979). Reactive oxygen metabolites discharged by inflammatory cells (Freeman and Crapo, 1982; Bowern *et al.*, 1984; Hartung *et al.*, 1988) that predominate in acute EAE lesions and other humoral factors (Lisak, 1980; Prineas and Graham, 1981; Wisniewski *et al.*, 1985; Sergott *et al.*, 1988) may contribute to demyelination both in EAE and MS. In acute EAE these agents may affect the myelin sheath (Itoyama and Sternberger, 1980) that is later phagocytozed, as in MS (Raine and Scheinberg, 1988). Alternatively, changes in axonal transport that supply myelin components (Toews *et al.*, 1988) may indirectly affect the myelin sheath in EAE. Whatever the mechanism, decreases of myelin sheath thickness and the total absence of the myelin sheath, classical segmental demyelination, are both attributes of the optic nerve ultrastructure of acute EAE lesions. While complete loss of the myelin sheath slows conduction (McDonald and Sears, 1970), reduced myelin sheath thickness may offer an explanation for the increases in the latency of the visual evoked potential in initial attacks of EAE with scant histopathological evidence of demyelination (Lidsky *et al.*, 1980; Hayreh *et al.*, 1981; Bilbool *et al.*, 1983) and cases of MS without visual symptomatology (Halliday *et al.*, 1972; Hume and Waxman, 1988). A substantial shift of the fibre spectra toward smaller diameters in comparison with the control animals was observed in the optic nerves of guinea pigs with EAE.

While the fibre spectra of a particular species may be influenced by the focality of demyelination in the optic nerve, the regional heterogeneity of fibres throughout the central and peripheral quadrants of the optic nerve of the guinea pig (Guy *et al.*, 1989*c*), similar to the rat (Reese, 1987), may have precluded such an effect. In contrast, the predominance of smaller diameter fibres situated centrally in the optic nerve of the primate (Sanchez *et al.*, 1986), and temporal crescent of the cat (Williams and Chalupa, 1983) may skew the fibre spectra with demyelination, primarily attacking small fibres when localized to these regions. If similar logic is applied to man, demyelination of the centre of the optic nerve involving small and medium calibre fibres gives rise to a central scotoma, while disorders involving the peripheral portion of the nerve affecting large fibres result in arcuate and peripheral visual field defects (Miller, 1988). In the normal guinea pig, the heterogeneity of the fibre spectra, in central and peripheral quadrants, eliminated the effect of regional variations in fibre calibre on the distribution function of myelin sheath thickness in demyelinated nerves. We found that optic nerve fibres of all diameters were affected by acute EAE.

Reductions in the calibre of fibres may contribute to the temporal dispersion associated with the shift of the visual evoked potential to longer latencies (Halliday *et al.*, 1972; Lidsky *et al.*, 1980; Hayreh *et al.*, 1981; Bilbool *et al.*, 1983). Fibre calibre strongly influences conduction velocity, and although this is fastest in large fibres, such fibres make a relatively small contribution to the fibre population of the guinea pig optic nerve (Guy *et al.*, 1989*c*). Consequently, large fibres have a negligible impact on the mean fibre diameter in guinea pigs with EAE, but they have a substantial effect on slowing conduction in demyelinating disorders (Hall, 1967). While large diameter fibres are more susceptible to injury from elevations in intraocular pressure (Quigley *et al.*, 1987), we found fibres of all calibres were vulnerable to the adverse effects of EAE.

The relative stability of the mean g ratios for normal animals and those with acute EAE was surprising, since an increase in this ratio would have been expected in a demyelinating disorder, as is observed in the peripheral nervous system (Beuche and Friede, 1985).

The g ratio may be species dependent and genetically determined (Henry and Sidman, 1988), apart from the effect of primary demyelinating disorders. Examination of the axon diameter spectra revealed that the decrease in fibre diameter of demyelinated nerves was not solely due to reduced myelin thickness, but a decrease in axon diameter kept g from rising in harmony with the reduction in myelin sheath thickness. In acute EAE the axonal diameter spectra in the optic nerve were shifted towards smaller diameters in comparison with normal optic nerves. While the causal relationship of decreasing axonal size associated with reduced myelin thickness is unknown, it is perhaps an attempt by the neuron to adjust axon size to altered myelin thickness to near optimal values for conduction in a particular species (Bostock and Sears, 1976, 1978). In the absence of conspicuous evidence of myelin degradation products in the optic nerves, which would have indicated previous demyelination, the possibility of a contribution from a maturational impairment in myelination in the EAE animals has to be entertained. The animals were still growing at the time of the experiment. Although the increase in body weight was similar in both groups of animals, a selective effect of EAE on maturation of the nervous system is possible, whether this might be a selective effect on myelination or on increase in axon size with a secondary influence on myelination.

We are not the first to show a decrease in axon calibre associated with disorders of primary demyelination (Prineas and McLeod, 1976; Aguayo *et al.*, 1979). An increase in small diameter axons was observed in association with optic nerve demyelination induced by Semliki Forest virus by Tansey *et al.* (1985). They interpreted this increase of small calibre axons, less than 0.20 μm, to indicate the 'growth of axonal sprouts'. However, our computerized morphometric study illustrates that the frequency of reduction in axon calibre in the optic nerve is greater than can be appreciated by visual inspection and manual analysis of electron micrographs even by the most careful observers.

The mechanisms responsible for the reductions of axonal calibre are unclear. A decrease in slow axonal transport in demyelinated nerves (Rao *et al.*, 1981; Guy *et al.*, 1989*a*, *b*), reducing the delivery of microtubles and neurofilaments that comprise the axonal cytoskeleton, may play a role (Medori *et al.*, 1988). These reductions in axonal calibre may represent an adaptive neuronal response, perhaps in an attempt to preserve or restore electrical impulse conduction, albeit slowed in EAE (Lidsky *et al.*, 1980; Hayreh *et al.*, 1981; Bilbool *et al.*, 1983) and MS (Halliday *et al.*, 1972; Hume and Waxman, 1988). Reductions in axon size may conceivably have a beneficial effect on conduction in demyelinated nerves. Remissions in demyelinating disease are observed before remyelination (Davis, 1970; McDonald, 1974; Prineas and Connell, 1979; Smith *et al.*, 1982; Kaji *et al.*, 1988; Raine and Sheinberg, 1988). While the factors contributing to such recovery are unknown, Waxman *et al.* (1989) have shown unmyelinated axons of a small calibre in the neonatal optic nerve sustain conduction with the substantially lower density of sodium channels present in normally inexcitable internodal axolemma. If this effect occurs in EAE, conduction block due to the loss of action current along inexcitable internodes that prevents excitation at adjacent nodes of Ranvier in demyelinated nerves may be overcome by a substantially reduced axon calibre permitting conduction along the internodes of these small calibre axons. While plausible, this potential effect of axonal calibre reductions to conduction remains to be demonstrated for EAE.

Conduction velocity is dependent both on axon diameter (Gasser and Grundfest, 1939; Rushton, 1951; Ogden and Miller, 1966; Waxman and Swadlow, 1978; Cullheim and Ulfhake, 1979) and myelin properties (Minwegen and Friede, 1984). We found that both of these parameters were affected by EAE. A decrease in the calibre of optic nerve axons of EAE animals may help maintain some degree of conduction along normally inexcitable internodes, but the velocity of such conduction is diminished. Consequently, a decrease in axon calibre may contribute to the increase in latency of the visual evoked potential characteristic of experimental (Lidsky *et al.*, 1980; Hayreh *et al.*, 1981; Bilbool *et al.*, 1983) and human disorders of primary optic nerve demyelination (Halliday *et al.*, 1972; Hume and Waxman, 1988), particularly during the initial attack of EAE, in which classical segmental demyelination is not a prominent pathological finding.

Our study suggests a rationale for some of the apparent discrepancies between the electrophysiology and the neuropathology of human disorders and animal models of primary demyelination. The presence of naked axons in only a small percentage of fibres may not be sufficient for the marked slowing of conduction velocity associated with the demyelination of acute EAE. Although we did not perform electrophysiological studies, reduced myelin sheath thickness and reductions in axon calibre of the optic nerve may offer explanations for the slowing of conduction during the earlier phases of EAE (Lidsky *et al.*, 1980) and possibly in MS patients without visual symptoms. The relative stability of the g ratio in acute EAE, achieved through reductions in axon calibre, may play a role in the restoration of conduction in disorders of demyelination, albeit slowed.

ACKNOWLEDGEMENTS

Supported by a Merit Review Grant from the Veterans Administration Medical Center (J.G.), a grant from the National Multiple Sclerosis Society, New York (J.G.), and in part by an unrestricted grant to the Department of Ophthalmology from Research to Prevent Blindness, Inc., New York.

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(*Received December 20, 1989. Revised January 16, 1990. Accepted March 6, 1990*)

Brain (1991), 114, 295–307

MEMORY-CONTINGENT SACCADES AND THE SUBSTANTIA NIGRA POSTULATE FOR ESSENTIAL BLEPHAROSPASM

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SUMMARY

Essential blepharospasm and cranial dystonia are related focal dystonias of unknown aetiology. Blepharospasm induced by acute dopamine depletion in parkinsonism restricts saccade initiation possibly via the substantia nigra pars reticulata (SNpr). If essential blepharospasm and cranial dystonia similarly restrict saccades, then a selective, somatotopically arranged pathway such as the SNpr may be involved. To test this possibility memory-contingent and visually-guided saccades were measured in patients with essential blepharospasm and cranial dystonia. The latency of both forms of saccades was either significantly prolonged or excessively variable, while the accuracy and peak velocity of these fast eye movements were similar to age-matched control subjects. Essential blepharospasm and cranial dystonia alter the initiation of saccadic eye movements. Subcortical brain regions or pathways where eyelid, saccade and cranial/cervical motor control are somatotopically approximated, such as the SNpr, may be involved in blepharospasm.

INTRODUCTION

Blepharospasm is an involuntary movement disorder that produces intermittent periods of sustained or clonic eyelid closure. When it occurs without an identified disease process, it has been called essential blepharospasm. In some individuals essential blepharospasm slowly spreads beyond the eyelids, to involve oromandibular, cervical and occasionally laryngeal musculature and is then called cranial dystonia. The process is usually somatotopically restricted to these muscle groups and rarely spreads to produce generalized dystonia or involve the lower extremities (Meige, 1910; Marsden and Sheehy, 1982; Jankovic and Ford, 1983; Fahn, 1988).

The causes of essential blepharospasm and cranial dystonia are unknown. Similar clinical signs occur in neurological diseases involving the basal ganglia or rostral brainstem (Jankovic and Ford, 1983; Keane and Young, 1985; Kulisevsky *et al.*, 1988) and stimulation of the basal ganglia in the cat can cause transient tonic eyelid closure (Dieckmann and Hassler, 1967). These observations have led to the postulate that essential blepharospasm is a focal dystonia caused by abnormal output from the basal ganglia.

Blepharospasm can also be induced by acute dopamine depletion in human parkinsonism caused by 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP) (Hotson *et al.*, 1986). This form of hypodopaminergic blepharospasm is assocated with a restriction of saccade metrics temporally related to the onset of eyelid closure. This attenuation of saccades

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or rapid eye movements may involve the substantia nigra pars reticulata (SNpr). The SNpr is associated with the initiation of saccades to visual and remembered targets via two sequential gamma-aminobutyric acid (GABA) inhibitory connections to the intermediate layer of the superior colliculus (Hikosaka and Wurtz, 1983*a*, *b*, *c*). When this inhibition is pharmacologically increased, saccades to visual and remembered targets are delayed, less accurate and slower, similar to the combined effect of blepharospasm and severe MPTP Parkinsonism (Hikosaka and Wurtz, 1985*a*, *b*).

Saccades to peripherally presented visual stimuli (visually-guided saccades) are considered reflexive (Melvill Jones, 1986; Crawford *et al.*, 1989). Their initiation involves both the frontal eye fields and the superior colliculus (Schiller *et al.*, 1980). While either structure is sufficient to initiate visually-guided saccades, the superior colliculus is particularly important for facilitating short-latency responses called express saccades (Fischer and Ramsperger, 1984; Schiller *et al.*, 1987). In contrast, saccades to remembered targets (memory-contingent saccades) are considered volitional (Melvill Jones, 1986; Crawford *et al.*, 1989). Volitional saccades often require an inhibition of reflexive eye movements followed by a cognitive oculomotor response. One volitional saccade paradigm requires the generation of a saccade of equal amplitude to a target step, but in the opposite direction, and has been called an antisaccade (Hallett, 1978). Isolated lesions in the human frontal lobes impair the generation of antisaccades but not visually-guided saccades (Guitton *et al.*, 1985). The memory-contingent saccade paradigm requires a delay in the initiation of a response followed by an eye movement to a previously presented target location (Hikosaka and Wurtz, 1983*a*, *b* or *c*). Memory-contingent saccades appear more vulnerable to selective disorders of the basal ganglia than visually-guided saccades (Carl and Wurtz, 1985; Crawford *et al.*, 1989). This hierarchy of reflexive and volitional saccades permits the selective measurements of different saccade generating systems such as the SNpr to superior colliculus system.

The output of the SNpr projects not only to the superior colliculus but is also distributed to the musculature involved in cranial dystonia (DeLong *et al.*, 1983). In contrast, skeletal musculature receives basal ganglia output mainly from the internal segment of the globus pallidus. The association of blepharospasm with basal ganglia disorders and a restriction of saccade responses, combined with the somatotopic distribution of cranial dystonia, suggests that these focal dystonias may involve the SNpr. If this proposal is true, then essential blepharospasm and cranial dystonia may also impair the metrics of memory-contingent saccades and, to a lesser extent, visually-guided saccades. In this study, the saccade latencies to remembered and visual targets were prolonged or excessively variable, while saccade velocity and accuracy was normal in essential blepharospasm and cranial dystonia.

METHODS

Subjects

Patients with essential blepharospasm or cranial dystonia were selected from volunteers contacted by the Benign Essential Blepharospasm Foundation, in Beaumont, Texas, or the botulinum toxin therapeutic research program at the Smith-Kettlewell Eye Research Institute in San Francisco, California. Each subject had findings of intermittent involuntary closure of the eyelids that involved active contraction of the orbicularis oculi-musculature bilaterally. Individual patients were excluded if they had (1) hemifacial spasm, eyelid apraxia, a multisystem neurological disorder, or atypical signs on examination; (2) a history consistent with a neuroleptic-induced movement disorder; (3) a history of a major medical disorder, neuro-ophthalmological disorder, or glaucoma; or (4) a best uncorrected acuity of 20/200 or worse.

Control subjects were age and sex-matched volunteers from a medical research institute and the surrounding community. The criteria for exclusion were the same as for the patients. Informed consent was obtained from all subjects.

Eye movement recordings and tasks

Horizontal right eye position was monitored with a SRI Dual Purkinje-Image eyetracker that had a linear range of ±8° and a noise level of less than 1 min of arc (Crane and Steele, 1985). The eye position signals were low-pass filtered (d.c., 100 Hz) and velocities were obtained by analog differentiation (Gould Biophysical Differentiator Amplifier, high cut-off 100 Hz, −3 dB). Eyelid closure or blinks were also monitored by the eyetracker. The stimulus position, stimulus onset and offset, eye position, eye velocity and the occurrence of eyelid closure or blinks were displayed on a rectilinear polygraph and recorded on magnetic tape for later processing.

Each experimental session included three types of trials. (1) Trials for calibrating the eye movement signals; (2) the visually-guided saccade task, in which saccades were elicited by steps between illuminated visual targets; and (3) the memory-contingent saccade task, in which saccades were produced to remembered targets. Test sessions were less than 1 h long and frequent breaks were included to minimize fatigue.

The stimuli were produced with a series of 11 computer-controlled light-emitting diodes (LEDs) spaced 1.5° apart horizontally at a viewing distance of 114 cm. This stimulus was used to produce 3−15° target steps within the linear recording range of the eyetracker; 80% of the target steps were 7.5−15°. This unequal distribution of stimulus amplitudes was chosen because disorders of saccade accuracy and velocity may be more apparent with larger eye movements. The LEDs were behind partially transparent black gauze, the surrounding room environment was covered with black felt and the room appeared completely dark to the test subjects except for the LED lights.

In the visually-guided saccade task, individual LEDs were sequentially lit without an interval overlap or gap (fig. 1A). The subjects were instructed 'to keep their eye on the red dot as it jumped back and forth'. Each trial included 20 pseudorandom stimulus changes and trials were run until at least 25 horizontal saccades were recorded that were not contaminated by blinks or eyetracker errors.

The stimuli for memory-contingent saccades were also under computer control and were similar to previous reports (Hikosaka and Wurtz, 1983*b*). The task required the subject to remember and subsequently look at the position of an LED that was illuminated for 100 ms while fixating on another LED (*see* fig. 1B for full timing sequence). Both control subjects and patients demonstrated an initial tendency to produce premature saccades to the 100 ms flashed target. Therefore, two instructions were particularly stressed. First, the subjects were told not to look at the flashed target but to keep looking at the fixated target until it went off, meanwhile remembering the location of the flashed target. Secondly, the subjects were instructed to 'quickly' make a saccade in darkness to the remembered target location when the fixated target went off. Initial training trials without eye movement recordings were performed to facilitate subject understanding and to minimize premature saccades. Each trial included 10 saccades to remembered locations and trials were run until at least 25 saccades uncontaminated by eye blinks were recorded

Data analysis

The recorded signals were digitized and sampled at a rate of 500 Hz with a 12-bit A/D converter, and an interactive computer program was used for off-line saccade analysis. The onset of each saccade task within a trial was identified and the stimulus position, eye position, eye velocity and eye closure recordings over the duration of the task were displayed on a graphics terminal. The initiation of the visually-guided or memory-contingent saccade was identified using acceleration criteria (Boman and Hotson, 1988). The initial and final eye positions and peak velocity of each saccade were automatically marked. Corrective saccades at the end of the memory-contingent tasks were identified by the experimenter and the relevant events were automatically marked. When necessary, these markings would be changed by the experimenter. Once the markings were correct, stimulus amplitude and saccade latency, amplitude, and peak velocity were computed and stored.

For each subject the means and SDs of the latencies and gains for visually-guided, memory-contingent and corrective saccades were computed. Saccadic gain was computed by dividing saccade amplitude by the stimulus amplitude. Amplitude-velocity curves for 3 – 15° saccades were derived and the peak velocity for 10° saccades was estimated using previously described methods (Hotson *et al.*, 1986). The measurements for right and left saccades were combined. The individual results from the patients and the control subjects were separately compiled and average saccade metrics were computed for each group. Both the means and SDs of the individual eye movement measurements were compared. The SD is a measure of the variability of the response and can be more sensitive in identifying a significant difference between groups than the mean value (Kowler and McKee, 1987). The Mann-Whitney U test was used for statistical comparisons between groups.

RESULTS

Subjects

Ten patients with essential blepharospasm, 5 patients with cranial dystonia and 15 age and sex-matched control subjects were selected and successfully completed the tests. All patients had chronic blepharospasm (Table 1). All but 1 patient stated that their blepharospasm was severe enough to impair visually-dependent tasks and therefore most had sought treatment with botulinum toxin or a myectomy. One patient was being treated with trihexyphenidyl and clonazepam at the time of the recording. The current botulinum toxin treatments were uniformly effective but with variable intervals between injections. These variations in active and past treatments precluded a valid quantification of the severity of blepharospasm and its subsequent correlation with saccade metrics. All patients and control subjects had a normal clinical examination of the visual fields, pupils, ocular motor responses, visual fixation, cover test, and had normal motor control in the limbs and a normal gait.

None of the patients treated with botulinum toxin injections before their eye movement recordings developed diplopia or other evidence of spread of the toxin to the extraocular muscles. All patients were studied at least 1 month after a toxin injection. Attempts to record remembered saccades immediately prior to the botulinum injections or in untreated patients with a similar severity of blepharospasm were technically unacceptable due to interference from uninhibited blinking or eyelid spasms.

TABLE 1 PATIENTS AND CONTROL SUBJECTS

	Bleph	*Dystonia*	*Total*	*Control*
Age (yrs)	57	57	57	56
(SD)	(7 8)	(9 9)	(8 2)	(7.2)
Acuity	20/64	20/96	20/75	20/86
(SD)	(46)	(67)	(55)	(85)
Female/male	6/4	3/2	9/6	9/6
Duration (yrs)	7.5	11.4	8 8	
Range	(2 – 18)	(3 – 25)	(2 – 25)	
Botulinum treatment	7/10	4/5	11/15	
Myectomy	2/10			

Bleph = essential blepharospasm, Dystonia = cranial dystonia; Total = essential blepharospasm plus cranial dystonia.

Intrasubject differences between visually-guided and memory-contingent saccades

When individual subject's visually-guided and memory-contingent saccades were compared, several differences were consistently found (fig. 1, Tables 2, 3). For both the patients and control subjects, the means and SDs of the latencies for memory-contingent

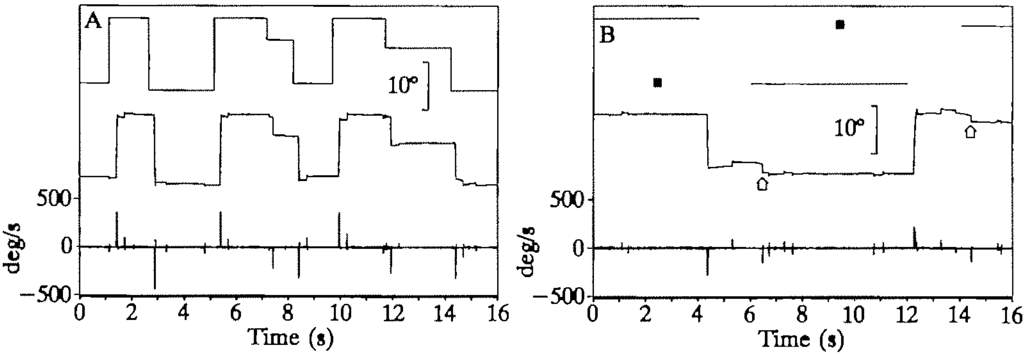

FIG 1. Typical examples of (A) visually-guided and (B) memory-contingent saccade recordings. In both A and B the *upper trace* indicates the stimulus position, the *middle trace* is eye position and the *lower trace* is eye velocity A, in the visually-guided saccade task the stimulus was always present and was switched between single LEDs every 1–4 s with a pseudorandom interval and location. The majority of target steps elicited saccades that crossed the midline and had a predictable direction. B, the memory-contingent saccade task began with the subject fixating on LED. An auditory cue was given and 500 ms later a second LED was illuminated for 100 ms while the subject maintained fixation on the first. The mark * indicates the position of the flashed LED that pseudorandomly varied between 10 possible locations. The initial fixation light went off 1.5–2 5 ms after the offset of the 100 ms duration LED and the subject looked to the remembered location of this flashed target while in darkness. The gaps in the *upper stimulus trace* indicates the duration of darkness. After another 2 s, the previously flashed LED was illuminated and the subject made a corrective saccade (arrows) if necessary

TABLE 2 VISUALLY-GUIDED SACCADES

	Bleph.	*Dystonia*	*Total*	*Control*
Latency	256*	252*	255**	215
(SD)	(41)	(34)	(38)	(27)
Gain	0.91	0.82	0.89	0.92
(SD)	(0.06)	(0.14)	(0.12)	(0 04)
Velocity	345	384	358	384
(SD)	(31)	(72)	(49)	(59)
Mean of SDs				
SD latency	68	71*	69	48
(SD)	(39)	(21)	(33)	(16)
SD gain	0.13	0 14	0.13	0 11
(SD)	(0.06)	(0 04)	(0.05)	(0.05)

* $P < 0\ 05$, ** $P < 0.01$. In Tables 2–5 two-tail P values are for differences between the 3 patient groups (Bleph. Dystonia, Total) and the control group. The latency (ms), gain and velocity (deg/s) measurements, are group means with standard deviations (SD). The mean of SDs is a measurement of group variability It is the mean of individual latency and gain SDs from each patient or control subject.

TABLE 3. MEMORY-CONTINGENT SACCADES

	Bleph.	*Dystonia*	*Total*	*Control*
Latency	379	442*	400	360
(SD)	(51)	(66)	(62)	(54)
Gain	1.10	1.02	1.08	1.03
(SD)	(0.23)	(0.22)	(0.22)	(0.19)
Velocity	260	298	273	286
(SD)	(61)	(82)	(68)	(54)
Mean of SDs				
SD latency	120*	125*	121**	91
(SD)	(24)	(16)	(22)	(22)
SD gain	0.69	0.44	0.61	0.48
(SD)	(0.43)	(0.37)	(0.42)	(0.30)

* $P < 0.01$; ** $P < 0.001$.

saccades were consistently greater than for visually-guided saccades. Similarly, the estimated peak velocity of 10° memory-contingent saccades was lower than for visually-guided saccades in all subjects (Tables 2, 3).

The mean gain for these two types of saccades was similar although, on average, visually-guided saccades were mildly hypometric while memory-contingent responses were hypermetric (Ohtsuka *et al.*, 1989). The SDs of the gains for memory-contingent saccades, however, were much greater than for visually-guided saccades. Therefore, memory-contingent saccades in both patients and control subjects had longer and more variable latencies, were slower, and had more variable gains than visually-guided saccades (Crawford *et al.*, 1989).

Intergroup differences for visually-guided saccades

The latencies of visually-guided saccades were consistently prolonged in patients with blepharospasm and cranial dystonia when compared with control subjects (Table 2). Seven of 15 patients had mean saccade latencies that were more than 2 SDs higher than the mean value for control subjects. The variability of saccade latency as measured by individual SDs was similarly excessive in 6 of 15 patients. The latency variability was significantly excessive only in the patients with cranial dystonia and not in the patients with essential blepharospasm. There were no consistent differences, however, between the groups when saccade gains and velocity estimates were compared. Therefore, the latencies of visually-guided saccades, but not their accuracy or velocity, were altered in patients with blepharospasm and cranial dystonia.

Intergroup differences for memory-contingent saccades

When the matrics of the memory-contingent saccades of patients and control subjects were compared, again it was found that only the latencies were significantly different (Table 3). The mean saccade latency of patients with cranial dystonia, but not essential blepharospasm, was significantly prolonged when compared with control subjects. In total, 4 of 15 patients had mean latencies greater than 2 SDs from the control subjects' mean value. The variability or individual SDs of saccade latencies was significantly excessive both in the patients with essential blepharospasm and cranial dystonia.

In contrast to saccade latencies, the gains and the peak velocities of memory-contingent saccades were similar in blepharospasm patients, cranial dystonia patients and control subjects. Therefore blepharospasm did not cause the extensive impairment of memory-contingent saccades that has been observed in primates with chemical lesions involving the SNpr output (Hikosaka and Wurtz, 1985*a*, *b*) or in humans with hemiparkinsonism (Carl and Wurtz, 1985).

Selective impairment of memory-contingent saccades

Memory-contingent saccades are usually slower, have more variable gains, and have longer and more variable latencies than visually-guided saccades. These differences are exacerbated by chemical lesions that disrupt the output of the SNpr (Hikosaka and Wurtz 1985*a*, *b*). If blepharospasm reflects a disorder of neural transmission mediated through the SNpr, then it may also cause a selective disorder of memory-contingent saccades. To test this possibility further, the differences between the mean metrics of memory-contingent and visually-guided saccades were calculated for each patient and control subject and these mean differences were then compared across groups.

The mean differences between the metrics of saccades to remembered and visual targets, including the differences between SDs, were similar for the patients with blepharospasm and cranial dystonia and the control subjects (Table 4). There was no evidence of a selective impairment of memory-contingent saccades in patients with blepharospasm.

Our results show that visually-guided saccades in patients with essential blepharospasm have *one* significant difference from control subjects, a prolonged latency duration. The results also indicate that memory-contingent saccades in essential blepharospasm also have *one* significant difference, an excessive latency variability. In contrast, both visually-guided and memory-contingent saccades in patients with cranial dystonia have *two* significant differences, a prolonged latency duration and an excessive latency variability. Therefore both essential blepharospasm and cranial dystonia alter both forms of saccades.

TABLE 4 REMEMBERED AND VISUAL SACCADE DIFFERENCES

	Bleph.	*Dystonia*	*Total*	*Control*
Latency	145	185	159	145
(SD)	(55)	(59)	(60)	(59)
Gain	0.34	0.19	0.29	0.11
(SD)	(0.30)	(0.25)	(0.29)	(0.21)
Velocity	96	88	87	97
(SD)	(45)	(21)	(28)	(39)
Mean of SDs				
SD latency	51.9	54.6	52.8	42.8
(SD)	(38.7)	(27.5)	(34)	(27)
SD gain	0.61	0.28	0.50	0.37
(SD)	(0.39)	(0.30)	(0.38)	(0.29)

The findings with cranial dystonia are more prominent perhaps reflecting a greater severity or somatotopic extension of this disorder.

Corrective saccades

Memory-contingent saccades usually overshot or undershot the actual target position. Therefore, when the target was reilluminated at the end of the memory-contingent task, subjects usually produced a small corrective saccade (fig. 1). Corrective saccade latencies and amplitudes were determined and the amplitude was divided by the amplitude of the preceding target step to provide a corrective saccade gain (Table 5). This gain reflected the accuracy of the sustained eye position in darkness. In contrast, memory-contingent saccade gain reflected the accuracy of the initial saccade, but not the subsequent saccades or eye drifts that occurred in darkness.

The latencies for corrective saccades were consistently longer than the latencies of visually-guided saccades (Table 5). During the visually-guided saccade task there was always an expectation of a target step in a new location. In the memory-contingent saccade task the main instruction was to look at the location of a remembered target. When the stimulus location was reilluminated the subject first had to determine if a corrective saccade was necessary. The relatively long latencies for corrective saccades may reflect this extra step in saccade processing.

When comparisons were made between the metrics of corrective saccades by blepharospasm patients, cranial dystonia patients and control subjects, no significant differences were found (Table 5). In contrast to other saccade responses, neither the mean nor the SD of the latencies were significantly different between groups.

TABLE 5 CORRECTIVE SACCADES

	Bleph.	*Dystonia*	*Total*	*Control*
Latency	323	341	329	359
(SD)	(104)	(85)	(95)	(90)
Gain	0.14	0.11	0.13	0.12
(SD)	(0.04)	(0.05)	(0.05)	(0.03)
Mean of SDs				
SD latency	113	172	133	134
(SD)	(49)	(78)	(64)	(37)
SD gain	0.08	0.07	0.08	0.08
(SD)	(0.02)	(0.04)	(0.03)	(0.03)

Inhibition of premature saccades

In the memory-contingent saccade task, subjects were instructed not to look at the flashed target and to only make a saccade after the fixated target was extinguished. However, it took practice and feedback before subjects could consistently inhibit the tendency to look towards the 100 ms flashed target. Premature saccades are not prominent in the monkey's responses to a 50 ms target duration (Hikosaka and Wurtz, 1985*a*, *b*). In human control subjects, however, they can occur in 30% of the responses to a 200 ms flashed target (Crawford *et al.*, 1989). Therefore a restricted ability to inhibit these premature saccades is another problem that can be uncovered in the memory-contingent saccade task.

Each group was equally successful at inhibiting premature responses to the 100 ms flashed target. On the average, patients and control subjects produced a premature response for every 10 memory-contingent saccade trials. This finding also indicated that both the patients in each group and control subjects clearly understood the task and were equally attentive throughout the tests.

DISCUSSION

Visually-guided and memory-contingent saccades in essential blepharospasm and cranial dystonia had prolonged or excessively variable latencies, but normal accuracy and velocity. By contrast, the latency and accuracy of subsequent corrective saccades to visual targets were not affected in these disorders. Therefore focal dystonias involving eyelid and cranial musculature can disrupt the initiation of saccades in tasks that require disengagement of active visual fixation.

The variation in symptom duration, course and treatment precluded a correlation of saccade metrics with blepharospasm severity beyond separating groups into essential blepharospasm and cranial dystonia. Visually-guided and memory-contingent saccade latencies were both significantly increased and excessively variable in patients with cranial dystonia. In patients with essential blepharospasm, however, the latency findings were less consistent; visually-guided saccade latencies were prolonged but not excessively variable, while memory-contingent saccade latencies had increased variability but normal duration. The inclusive latency abnormalities found only with cranial dystonia may reflect the increased severity or somatotopic extension of this focal dystonia compared with essential blepharospasm.

There are several ways in which saccade initiation can be impaired. The responses could be altered by visual input, attention, central ocular motor processing or peripheral motor conduction. Each of these possibilities was considered.

The selective disruption of saccade initiation was not caused by restricted visual input due to involuntary eyelid closure or blinking. The Purkinje-Image eyetracker accurately monitors eyelid closure and records that were contaminated by these movements were excluded from the study. Also, most patients were successfully treated with botulinum toxin injections at the time of the eye movement recordings in order to avoid the problem of involuntary eyelid closure. There was therefore no reason to believe that the delayed reaction times were due to eyelid closure interfering with visual perception.

An increase in saccade reaction times could indicate an impairment in sustained attention on a task. The patient groups and control subjects, however, had a similar ability to suppress premature saccades towards the flashed target of the memory task. This suppression required sustained attention and effort to inhibit a natural tendency to look at the peripherally flashed targets (Crawford *et al.*, 1989). The blepharospasm patients could also make corrective saccades as quickly as the control subjects and they could similarly locate the position of the remembered target in the memory-contingent saccade task. Differences in sustained attention therefore did not explain the delayed or variable saccade latencies associated with blepharospasm.

Most of the patients had been repeatedly treated with botulinum toxin. Local injection of this toxin into the eyelids can spread to involve extraocular muscles and, when clinically symptomatic, produce diplopia. None of our patients had developed diplopia in association with their most recent eyelid injections. Local injection of botulinum toxin can also affect neuromuscular transmission in muscles remote from the site of injection; however, motor conduction velocities and distal motor latencies remain normal (Lange *et al.*, 1987). Even in severe systemic botulinum toxin poisoning, there is no abnormal delay in motor conduction or latencies (Gutmann and Pratt, 1976). Therefore, if saccade abnormalities were due to local or remote effects of botulinum toxin, then accuracy and velocity of both visually-guided and memory-contingent saccades should have been affected, not latency (Hedges *et al.*, 1983).

These observations suggest that the variable or prolonged saccade latencies seen in essential blepharospasm and cranial dystonia are not due to disorders of visual input, attention or peripheral ocular motor conduction. We therefore propose that these focal dystonias delay saccade initiation by disrupting central ocular motor processes. Brain disorders can impair the synkinesis between eyelids and saccades. For example, diseases involving the brainstem-cerebellar systems produce abnormalities of saccade metrics that are influenced by eyelid blinks and eye blinks may normally facilitate the initiation of saccades (Zee *et al.*, 1983). Our results indicate that focal dystonia or spasm of the eyelid can impair saccade initiation in tasks that require disengagement of active visual fixation.

In the patients with essential blepharospasm or cranial dystonia there was no evidence of a selective disorder of memory-contingent saccades. In fact with just blepharospasm, memory-contingent saccades were normal except for a significantly increased variability in response latency. Direct support for the postulate that blepharospasm and cranial dystonia is a disorder mediated via the SNpr output of the basal ganglia is therefore lacking. Also, in patients with mild to moderate Parkinson's disease, memory-contingent saccades may have reduced velocities (Carl and Wurtz, 1985) or accuracy (Crawford *et al.*, 1989). A significant prolongation of remembered saccade latency has not been a consistent finding in these patients with basal ganglia disorders. Furthermore, there are multiple brain areas that are related both to saccade processing and eye blinks. As noted above, cells related to memory-contingent and visually-guided saccades and eyelid closure have been found in the monkey basal ganglia (Hikosaka and Wurtz, 1983*a, b, c*; Dieckmann and Hassler, 1967). However, it is unclear whether eyelid blinks are directly influenced by the SNpr. Saccades and eyelid blinks can also be elicited from the frontal eye fields (Bruce and Goldberg, 1985), the frontal dorsomedial cortex (Schlag and Schlag-Rey, 1987), the pontine reticular formation (Cohen and Feldman, 1968) and the inferior parietal cortex (Shibutani *et al.*, 1984; Keating and Gooley, 1988). These brain regions may also influence the motor control of cranial-cervical musculature disturbed in cranial dystonia. Our results therefore link essential blepharospasm and cranial dystonia with a disorder of saccade systems, but not specifically with the SNpr to superior colliculus pathway.

However, when our eye movement results are combined with blink reflex studies in essential blepharospasm and cranial dystonia, the SNpr postulate can be indirectly supported. Studies of the blink reflex in blepharospasm using paired conditioning and test stimuli have consistently demonstrated that the late R_2 component is selectively enhanced (Berardelli *et al.*, 1985; Tolosa *et al.*, 1988). The R_2 response of the blink reflex is mediated by brainstem interneurons that either have a lower threshold for excitation or are hyperactive in blepharospasm. Parkinson's disease also selectively enhances the R_2 interneuron component of the blink reflex making it more resistant to habituation by repetitive stimulation (Kimura, 1973; Agostino *et al.*, 1987). In contrast, lesions of the sensorimotor cortex have the opposite effect and depress the blink reflex (Berardelli *et al.*, 1983; Kimura *et al.*, 1985). These findings plus clinical observations (Jankovic and Ford, 1983) make a cortical cause of blepharospasm unlikely. Since saccade gain and velocity are not altered in blepharospasm, a disorder of the saccade generators in the pontine reticular formation is also unlikely (Henn *et al.*, 1984; Hanson *et al.*, 1986). Also, chronic human frontal cortex lesions, unlike the monkey, may not alter the latency of remembered saccades or visually-guided saccades to unpredictable targets (Deng *et al.*, 1986; Fletcher and Gellmann, 1989). Our selective impairment of saccade latency or variability in conjunction with the blink reflex studies therefore adds further evidence that essential blepharospasm and cranial dystonia may be produced by a disorder of communication between the basal ganglia and the rostral brainstem. Since saccades are involved, it remains possible that the SNpr is also involved, perhaps selectively.

ACKNOWLEDGEMENTS

This work was supported by grant EY03387 from the National Eye Institute and a grant from the Benign Essential Blepharospasm Research Foundation. Dr Alan Scott generously contacted patients for this study.

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(Received November 10, 1989. Revised February 16, 1990. Accepted March 14, 1990)

Brain (1991), **114**, 309–320

CHANGES IN FAST AXONAL TRANSPORT OF GLYCOPROTEINS IN OPTIC NERVES OF MICE INFECTED WITH SEMLIKI FOREST VIRUS

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SUMMARY

[^{3}H]fucose incorporation into mouse retinae and subsequent axonal transport of glycoproteins to optic nerve terminals was studied before and during optic nerve demyelination induced by Semliki Forest virus (SFV) infection. As was previously found for [^{3}H]proline-labelled protein, axonally transported glycoprotein was increased before demyelination. Fluorographic analysis of the increased glycoproteins and proteins, after separation by gel electrophoresis, showed particularly large increases in labelling of 2 glycoproteins (38.1 kDa and 45.0 kDa) and 3 protein (15.9 kDa, 23.8 kDa and 27.7 kDa) bands. These increases were due to host-cell rather than viral components. At the time of demyelination, however, [^{3}H]fucose incorporation into retinae of SFV-infected mice was significantly depressed, resulting in reduced label incorporation into axonally transported glycoproteins. Since glycoproteins play a role in axon-glia recognition and adhesion, the early changes in their axonal transport may contribute to the mechanism of demyelination.

INTRODUCTION

Viruses have increasingly been implicated in demyelinating disease in recent years (Koprowski *et al.*, 1985; Waksman and Reingold, 1986). Pathophysiological changes found in immunocompetent mice following intraperitoneal inoculation with Semliki Forest virus (SFV) indeed resemble those in patients with multiple sclerosis (Kelly *et al.*, 1982; Tremain and Ikeda, 1983; Ikeda and Tansey, 1986).

In this virus model of central nervous system demyelination, before the commencement of demyelination, fast axonal transport of protein in optic nerves to superior colliculi was significantly increased (Tansey and Ikeda, 1986). Furthermore, both the increase in axonal transport and demyelination did not follow SFV infection in T cell deficient nude mice (Jenkins *et al.*, 1988). These findings, together with the fact that maintenance of myelin and axon integrity depend on axonal transport (Droz, 1979; Bray *et al.*, 1981), led us to hypothesize that axonal transport changes may promote demyelination, perhaps due to abnormal expression of proteins involved in axon-glia interaction.

The glycoprotein component of axonal transport is of particular interest since many of these molecules are destined for incorporation into the axonal plasma membrane, where they may interact with surrounding myelin and play a role in axon-glia recognition and adhesion (McGarry *et al.*, 1985; Hampson and Poduslo, 1987). We still do not know, however, whether any changes in axonal transport of glycoproteins occur in optic nerves of SFV-infected mice.

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We therefore studied axonal transport of [^{3}H]fucose-labelled glycoproteins both preceding and at the height of demyelination. The study consisted of two parts. First, in order to determine the optimal times when any changes in glycoprotein transport resulting from SVF infection occur, time-courses of [^{3}H]fucose incorporation into glycoproteins in the retina, and their subsequent transport to optic nerve terminals, were established. Secondly, to characterize changes in axonally transported components, a fluorographic analysis of axonally transported glycoproteins in comparison with proteins, after separation by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS PAGE), was carried out.

METHODS

All reagents were of analytical or electrophoresis grade and were obtained from BDH Ltd, Essex, or the Sigma Chemical Co., Dorset, UK, except where stated

Animals and virus inoculation

Male Swiss/A$_2$G mice, aged 3–3.5 wks, of the St Thomas' Hospital Medical School strain were inoculated (i.p.) either with 0.1 ml of sterile phosphate-buffered saline containing 0.75% (w/v) bovine serum albumin (BAPS), or with the A7(74) strain of SFV in 0.1 ml BAPS. Before the experiments, the virus titre was determined by injecting 10-fold serial dilutions of SFV in 20 μl BAPS intracerebrally into 3–4 day-old suckling mice The virus injected into experimental mice was equivalent to a $10^{4.5}$ intracerebral LD$_{50}$, which causes death in suckling mice within 2–3 days of inoculation.

Intravitreal injections of radiolabels

Detailed procedures for the intravitreal injections performed in these axonal transport studies have been described previously (Pessoa and Ikeda, 1984; Tansey and Ikeda, 1986). These are therefore described only briefly, together with minor modifications applied in this study.

While under deep sodium pentobarbitone anaesthesia (50 mg/kg Sagatal; May and Baker Ltd, Essex), each mouse received an injection of radiolabelled glycoprotein or protein precursor into the vitreous of one eye. Intravitreal injections were administered either on postinoculation day (PID) 10 or on PID 18, i.e., 10 or 18 days after i.p. inoculation of SFV or BAPS. These times correspond, respectively, to at least 4 days before demyelination begins and to the period of demyelination (Chew-Lim *et al.*, 1977). The mice recovered from anaesthesia within 1 h

Glycoproteins were labelled with L-[5,6-^{3}H]fucose (50 Ci/mmol) and proteins with L-[2,3,4,5-^{3}H]proline (122 Ci/mmol) obtained at radioactive concentrations of 1 mCi/ml (Amersham International, Bucks, UK). For time-course studies of axonal transport, 1 μl intravitreal injections of the above radioactive precursor stock solutions were administered and the labelled tissues processed for liquid scintillation counting. To measure the distribution of radiolabel incorporation into glycoproteins and proteins after separation by SDS PAGE, higher radioactive concentrations of the above precursors were needed. Thus precursor stock solutions were lyophilized and dissolved in sterile saline at a radioactive concentration of 25 mCi/ml immediately before intravitreal injection of 2 μl of these concentrated solutions.

Time-course study of axonal transport

Following injections of stock radiolabelled precursor, the mice were killed after different survival periods between 8 and 48 h. Full details of the preparation of tissues for determination of radiolabel incorporation have been described previously (Tansey and Ikeda, 1986). Briefly, eyes, optic nerves and superior colliculi were rapidly removed and fixed in 10% (w/v) trichloroacetic acid. After thorough washing with water, the retinae were dissected out and all tissues were weighed when dry. These were next moistened with 20 μl water prior to solubilization in 0.3 ml Solusol tissue solubilizer (National Diagnostics Ltd, Bucks, UK). Finally, 5 ml Fluorosol scintillant (National Diagnostics Ltd) were added and radioactivity measured in a Rakbeta 1215 scintillation counter (LKB-Wallac) with quench correction

Specific radioactivities of retinae, optic nerves and superior colliculi required correction for background radioactivity derived from blood-borne label. This was achieved, for optic nerves and retinae, by subtracting the radioactivity found on the side contralateral to the injected eye from that found in the ipsilateral structures. On the other hand, since 97% of mouse optic nerve fibres terminate in the contralateral superior colliculus, labelling in the side ipsilateral to the injection was subtracted from the contralateral side.

Additional corrections were also found to be necessary. When fast axonal transport to the superior colliculus was abolished by colchicine, which did not affect incorporation of labels into the retina, some acid-precipitable radiolabel was still detected in the optic nerve of the injected eye, even after the above corrections for systemic labelling had been made (unpublished observations). This consistently amounted to 1% of the specific radioactivity of [^{3}H]fucose, and 2% of [^{3}H]proline, in the retina. Hence, these values were subtracted from optic nerve specific radioactivity to calculate the actively transported component. The presence of this radioactivity was apparently due to diffusion of label from the injected eye along the optic nerve (Haley *et al.*, 1979). Data from control and experimental mice were compared using Student's paired t test.

Fluorographic studies of glycoproteins and proteins

Superior colliculi were rapidly removed 18–48 h following intravitreal injections of the concentrated stock radiolabels and homogenized at 0° C with solubilization buffer to give a concentration of 2% (fresh tissue w/v), and stored at −20° C. The solubilization buffer consisted of 62.5 mM tris-chloride, pH 6.7; 2.5% (w/v) SDS; 10% (v/v) glycerol; 5% (v/v) 2-mercaptoethanol; 1 mM dithiothreitol; 0.002% (w/v) bromophenol blue. The following protease inhibitors were also included: 2 mM phenyl methyl sulphonyl fluoride and 4 mM Na_2 EDTA.

Discontinuous SDS PAGE of solubilized tissue was carried out in 7.5–15% linear gradient gels (0.75 mm thick) using methods based on those of Laemmli (1970) and O'Farrell (1975). Molecular mass markers used were [^{14}C]methylated proteins (range. 14.3–200 kDa) obtained from Amersham International. To ensure identical treatment of experimental and control samples, they were electrophoresed on the same gel at 20 mA/gel for 30 min (stacking) and 10 mA/gel for 4–5 h (resolving). Gels were then equilibrated with Amplify (Amersham International) and dried on paper for fluorography. To obtain a linear relationship between radioactivity and silver grain density (Laskey and Mills, 1975), Fuji RX or Kodak X-Omat AR film was preflashed sufficiently to give an increase in background absorbance of 0.1–0.2 units (A_{633}). They were then exposed to the dried gel for up to 40 days at −70° C and after development the resulting fluorographs were scanned with an LKB-Ultroscan XL laser densitometer. Data analysis was performed with the aid of LKB 2400 GelScan XL software which integrates the densitometer output and provides molecular weight calibration

RESULTS

Time-courses of axonal transport of glycoproteins on PID 10, before demyelination

Fig. 1 shows the time-courses of incorporation and transport of [^{3}H]fucose in retinae, optic nerves and superior colliculi in SFV-infected and control mice on PID 10. The label appeared at the same time in optic nerves and superior colliculi in both control and SFV-infected mice, suggesting that the rate of fast axonal transport of glycoproteins was not affected by SFV infection.

There were no significant differences in the labelling of glycoproteins between control and SFV-infected mouse retinae (fig. 1A), although the level of label incorporation did tend to be slightly higher in the SFV-infected mice than in controls. In both control and SFV-infected mice, the peak levels of [^{3}H]fucose labelling found in the optic nerves was approximately 5% of that incorporated into the retina (*cf.* figs 1A, B), whereas only about 1% of the [^{3}H]fucose incorporated into the retina was transported to superior colliculi (*cf.* figs 1A, C).

Glycoprotein labelling reached peak levels in superior colliculi of both SFV-infected and control mice 18 h after intravitreal injection. However, whereas incorporation of

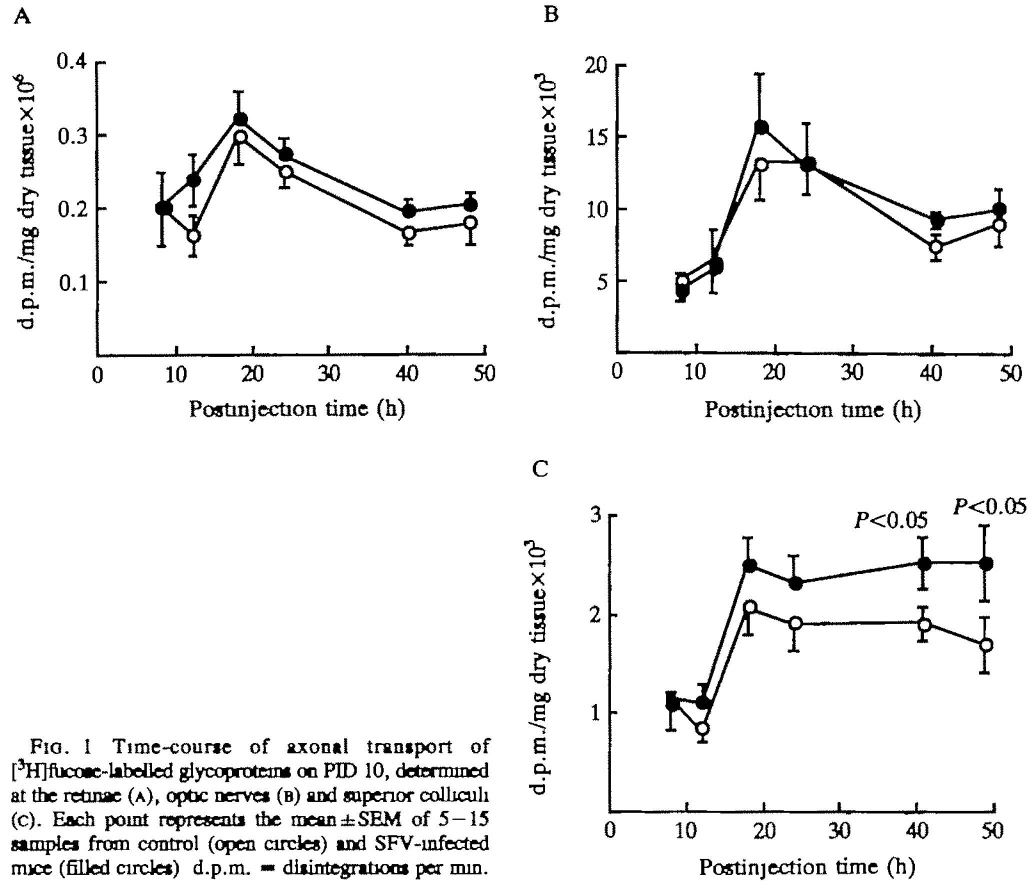

Fig. 1 Time-course of axonal transport of [^{3}H]fucose-labelled glycoproteins on PID 10, determined at the retinae (A), optic nerves (B) and superior colliculi (C). Each point represents the mean ± SEM of 5–15 samples from control (open circles) and SFV-infected mice (filled circles) d.p.m. = disintegrations per min.

label declined after 24 h in the control superior colliculi, glycoprotein levels remained elevated in SFV-infected mouse brain. Thus, 40–48 h after injection of [^{3}H]fucose, glycoprotein labelling in superior colliculi of SFV-infected mice was 33–49% higher than in controls ($P < 0.05$).

Time-courses of axonal transport of glycoproteins on PID 18 at the time of optic nerve demyelination

Fig. 2 shows the time-courses of incorporation and transport of [^{3}H]fucose-labelled glycoproteins in retinae, optic nerves and superior colliculi on PID 18. A 47% decrease ($P < 0.01$) in the level of [^{3}H]fucose incorporation into glycoproteins was seen in the retinae of SFV-infected mice compared with controls 18 h after injection of the label (fig. 2A). Consequently, [^{3}H]fucose incorporation in axonally transported glycoproteins was depressed by up to 67% ($P < 0.01$) in optic nerves (fig. 2B) and 56% ($P < 0.001$) in superior colliculi (fig. 2C) 18–24 h after intravitreal injection. Glycoprotein labelling in retinae, optic nerves and superior colliculi of control mice on PID 18 declined between 24 and 28 h after injection of [^{3}H]fucose, whilst that in SFV-infected mice remained depressed.

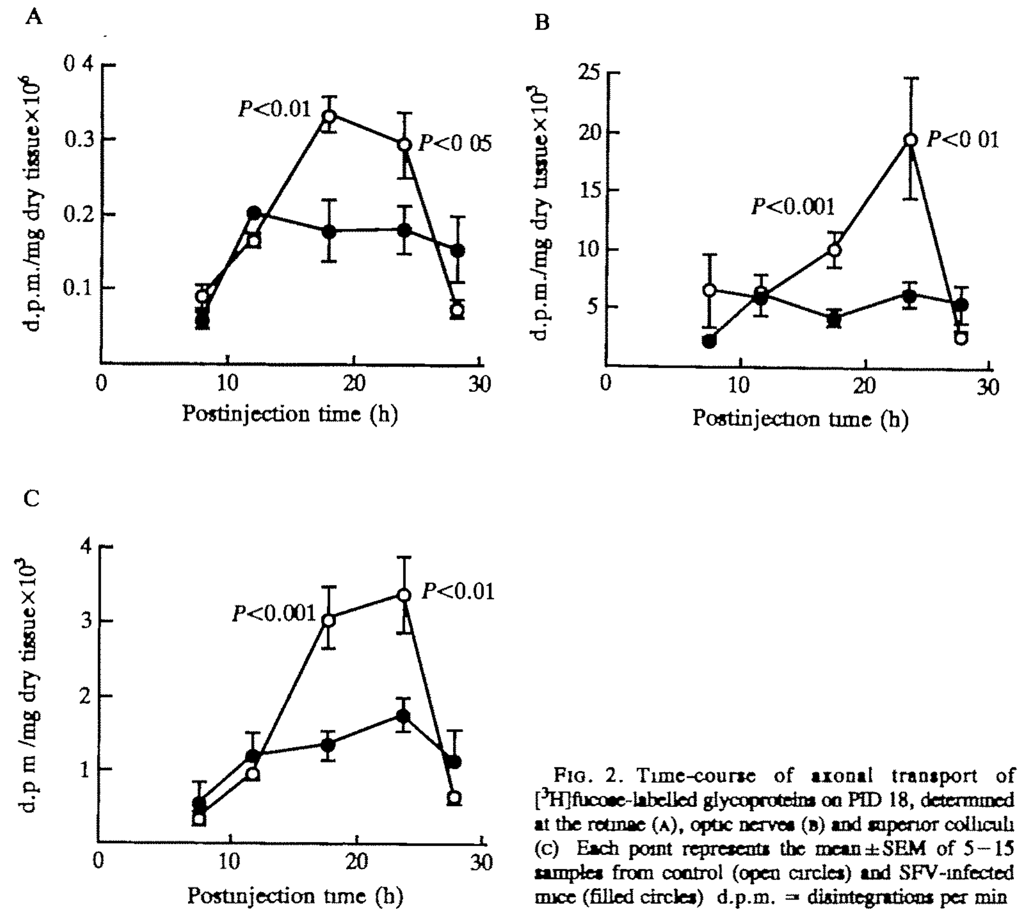

FIG. 2. Time-course of axonal transport of [^{3}H]fucose-labelled glycoproteins on PID 18, determined at the retinae (A), optic nerves (B) and superior colliculi (C) Each point represents the mean±SEM of 5–15 samples from control (open circles) and SFV-infected mice (filled circles) d.p.m. = disintegrations per min

Fluorographic analysis of axonally transported glycoproteins and proteins on PID 10 before demyelination

Fig. 3A shows a computed densitogram revealing increased labelling of glycoproteins from superior colliculi of SFV-infected mice, 48 h after intravitreal injection of [^{3}H]fucose on PID 10. Similarly, fig. 3B shows data obtained by the same method, corresponding to the previously reported increase in labelling of axonally transported proteins at optic nerve endings of SFV-infected mice (Tansey and Ikeda, 1986; Jenkins *et al.*, 1988), 18 h after intravitreal injection of [^{3}H]proline on PID 10. The densitograms were obtained by subtracting the mean absorbance values obtained from individual gel tracks containing control material from those corresponding to SFV-infected mice.

The total integrated absorbances of the glycoprotein and protein bands were increased by 32% and 85%, respectively, in samples from SFV-infected mice. These observations agree reasonably well with the liquid scintillation counting data, which showed increases in axonally transported glycoproteins and proteins of 49% (fig. 1c) and 60–80% (Tansey

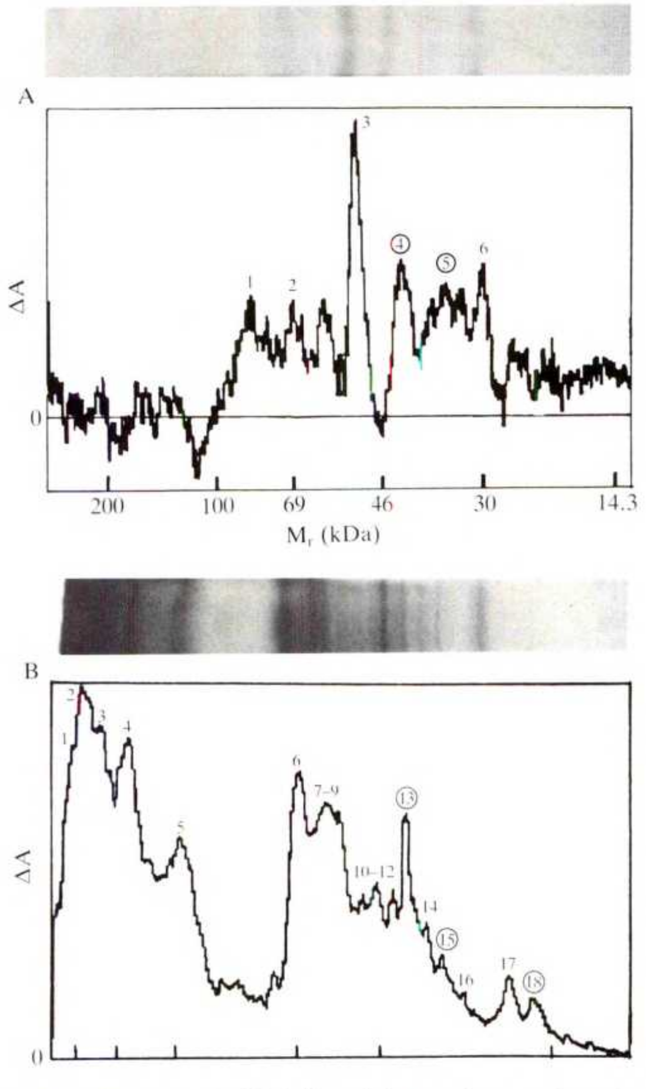

FIG. 3. Densitograms showing increased radiolabelling of glycoproteins (A) and proteins (B) in superior colliculi of SFV-infected mice on PID 10. The plots were obtained by subtracting the mean absorbance of 4 control fluorogram tracks from that of 5 tracks derived from SFV-infected brain, with the aid of LKB GelScan XL software. Samples of fluorogram tracks are shown above.

and Ikeda, 1986; Jenkins *et al.*, 1988) respectively. The glycoprotein bands were characteristically broad compared with proteins.

Table 1 shows the mean integrated absorbances ± SD of bands on fluorograms of gels containing axonally transported glycoproteins and proteins from superior colliculi of 4 control (BAPS) and 5 SFV-infected mouse brains on PID 10. Band numbers correspond to those shown in figs 3A and B. Several protein bands had M_r values below 31 kDa, whereas the lowest M_r found amongst the fucose-labelled glycoproteins was

TABLE 1 MEAN INTEGRATED ABSORBANCES ± SD OF BANDS ON FLUOROGRAPHS OF GELS CONTAINING AXONALLY TRANSPORTED GLYCOPROTEINS AND PROTEINS OBTAINED FROM SUPERIOR COLLICULI OF 4 CONTROL AND 5 SFV-INFECTED MICE ON PID 10[1]

		[^3H]fucose-labelled glycoproteins		
Band no.	*M_r (kDa)*	*Control*	*SFV*	*Increase (%)*
G1	124.0±9.0	0.83±0.81	0.95±0.20	15
G2	71.3±1.3	0.40±0.12	0.57±0.18	44
G3	53.4±0.8	0.44±0.06	0.54±0.16	24
G4	45.0±0.9	0.16±0.02	0.27±0.11	68*
G5	38.1±1.3	0.21±0.05	0.35±0.10	68**
G6	31.2±0.8	0.23±0.05	0.31±0.07	35*
		[^3H]proline-labelled proteins		
P1	≥200	3.33±1.07	3.87±0.99	16
P2	172 ±9	1.10±0.09	2.01±0.59	82***
P3	145 ±14	1.64±0.28	3.02±2.26	84
P4	95.0±6.0	0.95±0.26	2.10±0.64	121***
P5	69.5±2.0	0.81±0.28	1.54±0.56	90**
P6	47.4±1.7	0.47±0.23	1.30±1.19	177
P7	44.2±2.0	0.21±0.06	0.58±0.27	176**
P8	41.4±2.1	0.66±0.28	1.20±0.50	82*
P9	38.9±1.8	0.39±0.14	0.78±0.42	100*
P10	35.2±1.3	0.20±0.02	0.56±0.39	180*
P11	31.9±1.5	0.25±0.07	0.62±0.36	148*
P12	29.3±0.9	0.19±0.06	0.36±0.20	89
P13	27.7±0.7	0.36±0.16	1.10±0.69	206*
P14	25.7±0.7	0.24±0.04	0.54±0.38	125
P15	23.8±0.9	0.15±0.03	0.47±0.25	213*
P16	21.8±0.8	0.10±0.03	0.23±0.15	130*
P17	17.7±0.3	0.17±0.07	0.44±0.29	159*
P18	15.9±0.3	0.07±0.01	0.21±0.11	200**

[1] Band numbers correspond to those shown in figs 1A and B * $P < 0.05$, ** $P < 0.025$, *** $P < 0.01$.

31.2 kDa. This is consistent with previous studies of axonally transported fucosylated proteins in rat brain (Goodrum *et al.*, 1979; Padilla and Morell, 1980*a*, *b*).

No newly expressed glycoprotein or protein bands were seen in superior colliculi in SFV-infected mice. Many glycoprotein and protein bands, respectively, showed increases in radiolabelling of around 30% and 100% in SFV-infected mice. However, 2 glycoprotein bands, G4 (45.0 kDa) and G5 (38.1 kDa) were increased by 68% ($P < 0.05$), while 3 protein bands with relatively low molecular weights, P13 (27.7 kDa), P15 (23.8 kDa) and P18 (15.9 kDa) showed striking increases ($P < 0.05$) of about two-fold.

Fluorographic analysis of axonally transported glycoproteins and proteins on PID 18 at the time of optic nerve demyelination

Fig. 4 shows a computed densitogram revealing decreased radiolabelling on a fluorogram of radiolabelled glycoproteins in superior colliculi of SFV-infected mice on PID 18. In order to produce positive absorbance peaks in the densitogram, the mean absorbance values of 5 gel tracks containing material from SFV-infected mice was

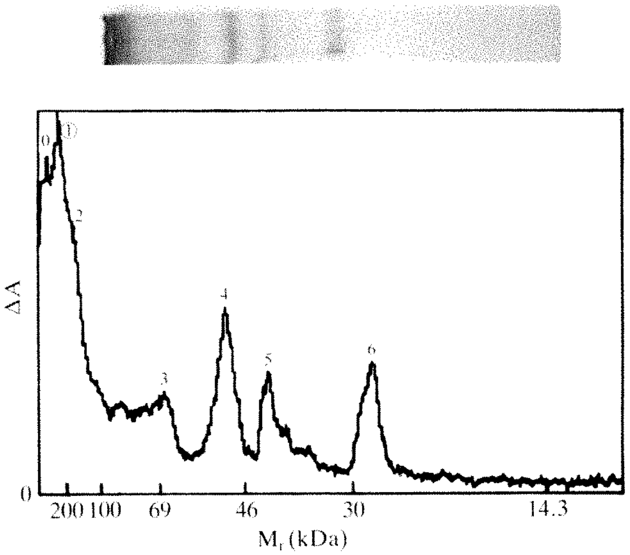

FIG. 4. Densitogram showing decreased radiolabelling of glycoproteins in superior colliculi of SFV-infected mice on PID 18. The plot was obtained by subtracting the mean absorbance of 4 control fluorogram tracks from that of 5 tracks derived from SFV-infected brain, with the aid of LKB GelScan XL software. A sample fluorogram is shown above.

TABLE 2. MEAN INTEGRATED ABSORBANCES ± SD OF BANDS ON FLUOROGRAPHS OF GELS CONTAINING AXONALLY TRANSPORTED GLYCOPROTEINS OBTAINED FROM SUPERIOR COLLICULI OF 4 CONTROL AND 5 SFV-INFECTED BRAINS ON PID 18[1]

		[^3H]*fucose-labelled glycoproteins*		
Band no.	M_r *(kDa)*	*Control*	*SFV*	*Decrease (%)*
0	>200	1.44 ± 0.51	0.75 ± 0.39	48*
1	>200	1.13 ± 0.21	0.65 ± 0.30	43**
2	170–200	1.05 ± 0.46	0.97 ± 0.57	8
3	68.6 ± 1.4	0.62 ± 0.25	0.40 ± 0.24	36
4	51.8 ± 0.9	0.93 ± 0.39	0.59 ± 0.29	37
5	43.5 ± 0.7	0.60 ± 0.19	0.46 ± 0.27	23
6	29.4 ± 0.7	0.59 ± 0.21	0.41 ± 0.20	31

[1] Band numbers correspond to those shown in fig. 4. * $P < 0.05$; ** $P < 0.025$.

subtracted from the 4 BAPS controls. The total integrated absorbance of glycoprotein bands from SFV-infected mice was reduced by 34% (fig. 2c), somewhat lower than the 56% decrease determined by liquid scintillation counting. On PID 18, no changes in the fluorographic pattern of axonally transported proteins were found in SFV-infected mice.

Table 2 lists the mean integrated absorbances ± SD of bands observed on the fluorogram containing axonally transported glycoproteins from superior colliculi of 4 control (BAPS) and 5 SFV-infected mice, 24 h after intravitreal injection on PID 18. Band numbers correspond to those shown in fig. 4. Although smaller decreases in the mean labelling of other glycoprotein bands were observed, with band no. 2 showing little change, glycoprotein band numbers 0 and 1 (>200 kDa) showed statistically significant decreases in [^{3}H]fucose labelling on PID 18 ($P < 0.025$).

DISCUSSION

Changes in glycoprotein transport before demyelination

We have previously shown that a significant increase in fast axonal transport of protein via optic nerves occurs prior to demyelination in SFV-infected mice (Tansey and Ikeda, 1986; Jenkins *et al.*, 1988). Our present enquiry as to whether axonally transported glycoproteins, which play an important role in axon-glia recognition and adhesion (McGarry *et al.*, 1985; Hampson and Poduslo, 1987), are also increased before demyelination proved affirmative.

Significant increases in the levels of axonally transported glycoproteins were found in superior colliculi of SFV-infected mice 24–48 h after injection of the label on PID 10 (fig. 1c). This period was substantially later than when increased levels of axonally transported proteins were found in the superior colliculi, i.e., 18 h after injection of the precursor (Pessoa and Ikeda, 1984; Tansey and Ikeda, 1986). The late appearance of this increase may represent more complex glycoprotein types, requiring extended processing in the Golgi apparatus (Specht and Grafstein, 1977; Goodrum *et al.*, 1979).

Many glycoprotein and protein bands from superior colliculi of SFV-infected mice showed increases in radiolabelling of similar magnitude (Table 1). Also, as was the case for proteins (Pessoa and Ikeda, 1984; Tansey and Ikeda, 1986), neither [^{3}H]fucose incorporation into retinae (fig. 1A), nor the rate of glycoprotein transport (fig. 1c), were significantly altered in SFV-infected mice. This indicates that SFV infection did not result in increased availability of precursor at the retina, but instead caused a stimulation of synthesis and glycosylation of axonally transported proteins. Thus a simple inflammatory response to virus infection, which could cause an increase in endothelial cell permeability (Pober, 1988), is unlikely to be the cause of the increased fast axonal transport that we observed.

Moreover, the stimulus for increased axonal transport found on PID 10 appears to be selective, since 2 glycoprotein bands (G4 and G5) and 3 protein bands (P13, P15 and P18) with relatively low molecular masses showed particularly large increases, whereas bands P1 and G1, with relatively high molecular masses, showed little change (Table 1). Furthermore, since the apparent M_r values of these bands did not correspond to those of known SFV proteins or glycoproteins (Lachmi and Kääriäinen, 1977; Bruce *et al.*, 1984), the changes in axonal transport seem to have occurred as a consequence of the immune response to viral infection, rather than as a direct effect of the virus itself. This observation is indeed consistent with our previous finding that neither axonal transport nor demyelination were observed in T cell deficient (athymic) nude mice when infected with SFV, despite enhanced virus replication (Jenkins *et al.*, 1988).

One component of axonal transport that might be altered in SFV-infected mice is neural cell adhesion molecule (NCAM), as this could lead to a disturbance of axon-myelin interaction. Indeed, protein band P2 (172 ± 9 kDa), which was increased by 82% (Table 1), could correspond to the 180 kDa NCAM. It has been shown that the 180 kDa NCAM is the predominant form expressed by neurons (Pollerberg *et al.*, 1985), and its deficiency has been demonstrated in the dysmyelinating quaking mouse mutant (Bhat and Silberberg, 1988). If altered axonal transport of NCAM-180 is a characteristic of genetically normal juvenile mice following SFV infection, the possibility that demyelination results from this is worthy of further examination.

We also considered that the changes in axonally transported molecules might include members of the heat shock protein family, which are expressed in response to several types of physiological stress. However, these seem to be carried by slow axonal transport (Clark and Brown, 1985). An increase in slow axonal transport of proteins has been shown to occur only concurrently with demyelination, and hence it is unlikely to be a cause of demyelination (Ikeda and Tansey, 1986).

Changes in glycoprotein transport at the time of demyelination

In contrast to the increased axonal transport before demyelination (PID 10), glycoprotein transport was reduced at the time of demyelination on PID 18 (fig. 2c). Changes in the level of fucosylation in retinae of SFV-infected mice on PID 10 were absent (fig. 1A), whereas retinal fucose incorporation on PID 18 (fig. 2A) was reduced by about 50%. Thus the reduction in axonal transport of glycoprotein was due to reduced fucosylation at the retina. The effects of such a decrease are difficult to predict without further information concerning the nature of the glycoproteins affected. It should be noted that the glycoproteins which showed decreased labelling on PID 18 were quite distinct from those which were increased on PID 10. The reduction in transport of glycoproteins cannot have been directly due to the virus itself since replication of SFV within the CNS ceases within a week after SFV inoculation (Illavia *et al.*, 1982; Pathak and Webb, 1988), and is cleared within 12 days (Jagelman *et al.*, 1978). It seems more likely that this change was a consequence of demyelination, since myelin loss occurs from PID 14 (Chew-Lim *et al.*, 1977).

Conclusion

In SFV-infected mice, axonal transport of glycoproteins in optic nerves, like proteins, was significantly increased before demyelination. On the other hand, at the time of demyelination, axonal transport of glycoproteins was decreased due to reduced fucosylation at the retina. These findings give further support to our hypothesis that abnormal increases in axonal transport may be a causative factor for demyelination. In addition, changes in the composition of axonally transported molecules are sustained well beyond the period of virus replication. Many glycoprotein and protein bands from the superior colliculus showed increases but the M_r values for these bands did not correspond to those of known SFV proteins or glycoproteins. This is consistent with our earlier findings that both axonal transport changes and demyelination are consequences of the immune response. Further analysis of axonally transported molecules using methods with greater resolution and sensitivity is necessary before we may identify the molecules that contribute to the changes found in this study.

ACKNOWLEDGEMENTS

This work was supported by grants from the Multiple Sclerosis Society of Great Britain and Northern Ireland and the Special Trustees of St Thomas' Hospital. We thank Edith Okorie and Eileen Ofori-Kuragu for technical assistance and Angela Dalton for secretarial assistance. We are grateful to Professor H. E. Webb of the Neurovirology Department, The Rayne Institute, for the gift of SFV, and Dr R. Wolstencroft of the Immunology Department, The Rayne Institute, for a critical reading of the manuscript.

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(*Received December 19, 1989. Accepted March 16, 1990*)

Brain (1991), 114, 321–332

TASK-DEPENDENT VARIATIONS IN PARKINSONIAN MOTOR IMPAIRMENTS

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SUMMARY

Studies of visually-guided arm movements in patients with Parkinson's disease (PD) have suggested a relationship between slowness of movement and a reduction in the ratio of movement amplitude and peak velocity. Recent studies indicate, however, that PD impairments may be different for well-learned, natural movements performed without visual guidance, or movements associated with sequential motor behaviours such as speech. To address this issue, PD subjects and age/sex-matched controls were compared on the performance of three tasks, all of which required lowering the jaw: (1) single, rapid, visually-guided movements; (2) equivalent movements associated with a single speech syllable (inherently without visual guidance), and (3) well-learned speech movements produced in a natural sequence. PD subjects manifested similar deficits for visually-guided jaw lowering as those previously reported for arm movements, namely reduced velocity/amplitude ratios and increased movement durations. By contrast, analogous jaw movements during the sequential speech tasks were unimpaired on these measures. These results suggest that PD motor impairments are influenced by a variety of factors, including the degree to which tasks are familiar and natural, and the availability of visual information.

INTRODUCTION

Kinematic studies of patients with Parkinson's disease (PD) have focused primarily on a limited variety of novel, visually-guided arm movements, typically yielding deficits of reduced velocity and prolonged duration (Draper and Johns, 1964; Flowers, 1975, 1976, 1978; Hallett and Khoshbin, 1980; Evarts *et al.*, 1981; Marsden, 1984; Accornero *et al.*, 1985; Sanes, 1985; Benecke *et al.*, 1986, 1987). Data gathered under such limited circumstances may not be good indicators of a patient's level of functioning (Berardelli *et al.*, 1986) and could promote an artificially simple view of motor deficits in PD. To this end, recent work indicates that motor disturbances observed in PD may vary both as a function of task and motor system (Abbs *et al.*, 1987; R. Inzelberg, T. Flash, E. Henis and A. D. Korczyn, unpublished; Connor *et al.*, 1989; *see also* Sanes and Evarts, 1985; Teasdale and Stelmach, 1988; Forrest *et al.*, 1989). For example, in well-learned, natural movements for speech, PD movement durations and ratios of peak velocity/movement amplitude were normal (Connor *et al.*, 1989; Forrest *et al.*, 1989), which is contrary to patterns typically reported in limb studies (Draper and Johns, 1964; Flowers, 1975, 1976, 1978). Neurophysiological mechanisms underlying these apparent task-related differences presumably include the somatotopic organization of basal ganglia

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structures and context-dependent neuronal functions (Pisa, 1988; *see also* Aldridge *et al.*, 1980; Alexander, 1987; DeLong and Alexander, 1987; Schneider, 1987).

Because there are anatomical and functional differences among limb and orofacial neuromotor systems (*see* Abbs *et al.*, 1987), the present study focused on motor task variations within a given orofacial muscle group. Our aim was to determine if movements of the jaw, performed by PD subjects (1) in an isolated speech syllable production, (2) as part of a natural speech sequence, or alternatively, (3) under visual guidance, independent of speech, manifested the same kinematic deficits.

METHODS

Subjects

Six idiopathic PD subjects and age/sex-matched controls (*see* Table) were studied. All subjects were paid for their participation and gave their informed consent according to the Declaration of Helsinki. All achieved at least 83% correct on a measure of cognitive function (Folstein *et al.*, 1975), passed a hearing screening test at 40 dB HL (0.5–4 kHz), and had normal or corrected vision. PD subjects were examined by a neurologist to rule out the presence of non-PD related dysfunctions. All PD subjects had mild speech impairments and were treated with appropriate dopaminergic enhancement medications. PD Case 4 was an exception; medication was discontinued 4 months before the study and a small resting jaw tremor was observed. Neurological and speech evaluations verified that normal subjects were free of any such impairments.

TABLE CLINICAL DATA FOR PARKINSONIAN SUBJECTS

Case	*Years postonset*	*Sex/age*	*Disability score**	*Medications*
P1	2	M-63	III	Sinemet
P2	3	M-77	II	Sinemet
P3	3	M-64	II	Sinemet
P4	2	M-59	II	None
P5	17	M-69	III	Bromocriptine Amantadine
P6	2	F-62	II	Sinemet Benztropine mesylate Bromocriptine

* Hoehn and Yahr (1967).

Apparatus and processing

A head-mounted cantilever beam instrumented with a strain gauge transducer was attached to the lower central incisors, providing a signal of superior-inferior movements of the jaw (*see* Barlow *et al.*, 1983). This signal was digitized at 15 bits (500 Hz) and then digitally low-pass filtered with zero phase lag using a fourth order Butterworth filter implementation (20 Hz cut-off). Instantaneous velocity and acceleration were then digitally derived with three-point numerical differentiation.

Tasks

The three tasks described below were completed by all subjects as part of a larger experimental protocol. Within each task, 'small', 'medium' and 'large' movement amplitudes were recorded. Subjects were instructed to perform each trial as quickly and accurately as possible for each task.

Visual task. Subjects faced a dual-trace oscilloscope 50 cm away at eye level displaying a fixed horizontal target marker (set at 4, 8, or 12 mm, relative to full jaw closure) and the jaw transducer output signal. The three specific target amplitude levels were chosen as representative of the range of speech movements reported elsewhere (Connor *et al.*, 1989), and did not approach the mechanical limits of jaw opening. Subjects were instructed to depress the jaw as quickly and accurately as possible after illumination of a stimulus light, thus superimposing the jaw signal and the target marker. Continuous monitoring of the oscilloscope traces was encouraged throughout the movement trial. After matching the two traces, subjects were instructed to elevate the jaw slowly and await the next stimulus light. Before experimental trials were initiated, subjects were trained to produce movement end-points within ±1 mm of target with 80% accuracy. A block of 30 experimental trials was collected at each of the 3 target amplitude levels, the order of target amplitudes was determined randomly. Exemplary jaw movements toward each target from 1 PD subject are shown in fig. 1A.

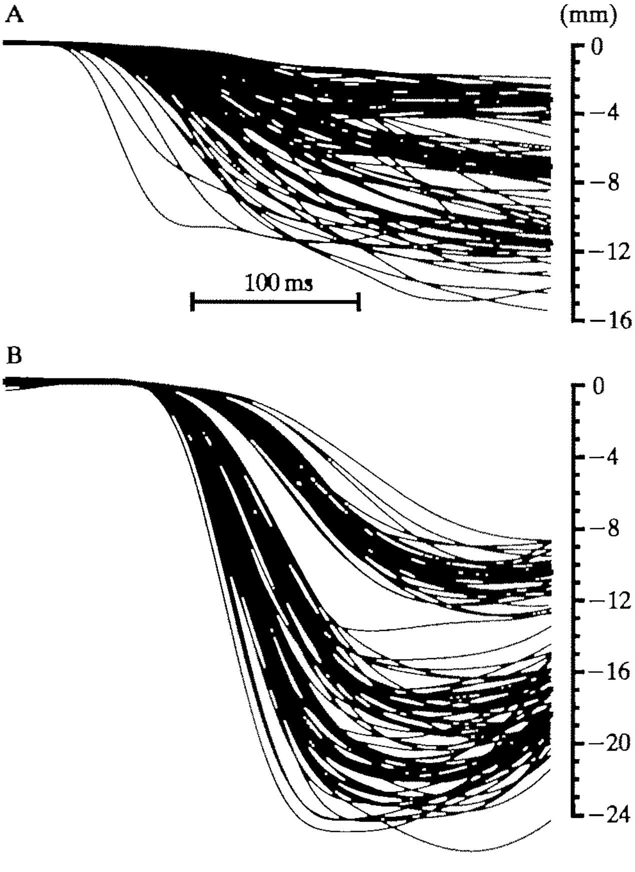

FIG. 1. A, visually-guided movements: jaw lowering trials from 1 PD subject (*Case 1*) towards 3 visual targets (4, 8, 12 mm) from a start position of full jaw closure ('0'). Thick lines represent the mean amplitude of each block of 30 trials. B, speech syllable movements: jaw lowering trials from 1 PD subject (*Case 3*) during 'da' at 3 loudness levels. Thick lines represent the mean amplitude of each block of 30 trials.

Speech syllable. Subjects were cued by a stimulus light to say the syllable 'da' at 1 of 3 loudness levels (i.e., self-determined conversational loudness, 10 and 20 dB above conversational), which were monitored by the examiner with a sound level meter. Training trials ensured that subjects maintained these loudness levels at ±2 dB for 'target' loudness. Jaw movement amplitudes naturally varied with these alterations in vocal intensity (fig. 1B). Feedback was provided to the subject as needed throughout each block of trials.

Speech phrase. A procedure identical to those used for speech syllables was employed to record the phrase 'Say da again' at the 3 different loudness levels. This yielded a range of jaw movement amplitudes for 'da' uttered within a more natural, sequential speech environment. Movements were qualitatively similar to those shown in fig. 1B.

The order of task presentation was counterbalanced across subjects. The amount of training provided to PD and normal subjects before each task condition did not differ significantly ($t = 1.08$, $P = 0.30$).

Measurement procedures

On a SUN Microsystems workstation, measurement intervals were marked with cursors at onset and completion of movement. These events were defined as 5% of the peak velocity associated with the initial acceleration and the first velocity zero-cross following attainment of the target (±1 mm), respectively. The following values were derived from the marked data by available computer programs: (1) movement duration, from movement onset to movement completion, (2) peak velocity magnitude; (3) maximum displacement amplitude; and (4) the ratio of peak velocity magnitude/displacement amplitude. Additionally, zero-crosses in acceleration were also automatically marked for each movement trial to demarcate the number of velocity peaks used in target attainment per trial.

Data analyses

To ensure that analyses consisted only of data where subjects understood task requirements and moved at near-maximum speeds, analyses were limited to blocks of trials following successful completion of training and for movements with durations less than 500 ms; 94% of normal and 90% of PD data met these two criteria. All speech data from PD Case 5, who evidenced no jaw movement during speech, were excluded from analysis. Movements manifesting more than 1 velocity zero-cross prior to target attainment were eliminated from the main statistical analyses, but were examined separately (*see* Velocity profile analysis in Results). Following these refinements, the resultant data set for the main statistical analyses consisted of 1280 PD movement trials and 1528 normal movement trials For each subject, averages were taken across trials within each condition and movement amplitude level. These averages were then used in the statistical analyses described below.

Two-factor ANOVAs (factors: (1) group: PD, normal; (2) amplitude: 'small', 'medium' and 'large') were performed to confirm experimentally-controlled differences in movement amplitude. Results indicated significant differences in amplitude for the 3 movement amplitudes in all jaw motor tasks. Movement amplitude levels were not different between groups for any movement task. However, when mean amplitudes among tasks were compared directly, the syllable task was found to have significantly greater amplitudes of jaw opening than the visual or phrase tasks (*see* fig. 1, for example). For this reason, further data analyses were performed only within each task with the aim of examining group differences, movement amplitude effects and their interactions.

Two-factor ANOVAs were then performed within each task for the experimental measures of movement duration and ratio of peak velocity/amplitude (*SPSS-X User's Guide*, 1988). Data were log-transformed prior to the ANOVAs and each data set approximated a normal distribution. Experimental error was controlled by setting individual ANOVA significance levels at 0.03 (Cohen and Cohen, 1975).

RESULTS

Task-dependent performance

Kinematics. PD subjects manifested different kinematic patterns for the three jaw motor tasks. When examined statistically within each task, PD subjects manifested performance differences (compared with normal) that were task-related, including movement duration (fig. 2) and peak velocity/amplitude ratios (fig. 3).

Specifically, while the durations of PD jaw movement were significantly longer than normal for visually-guided movements ($F_{group} = 8.02$, df(1,30), $P = 0.008$; $F_{amplitude} = \text{n.s.}$, $F_{group \times amplitude} = \text{n.s.}$), durations of PD jaw opening during speech phrases

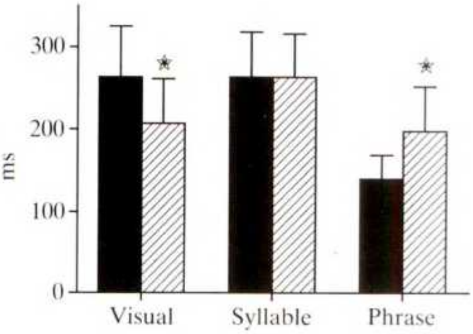

FIG. 2. Mean duration of movement for PD (filled bars) and normal groups (hatched bars) for jaw lowering toward visual targets, in a single syllable and within a phrase. Error bars represent 1 SD. Stars = significant group differences ($P \leq 0.03$).

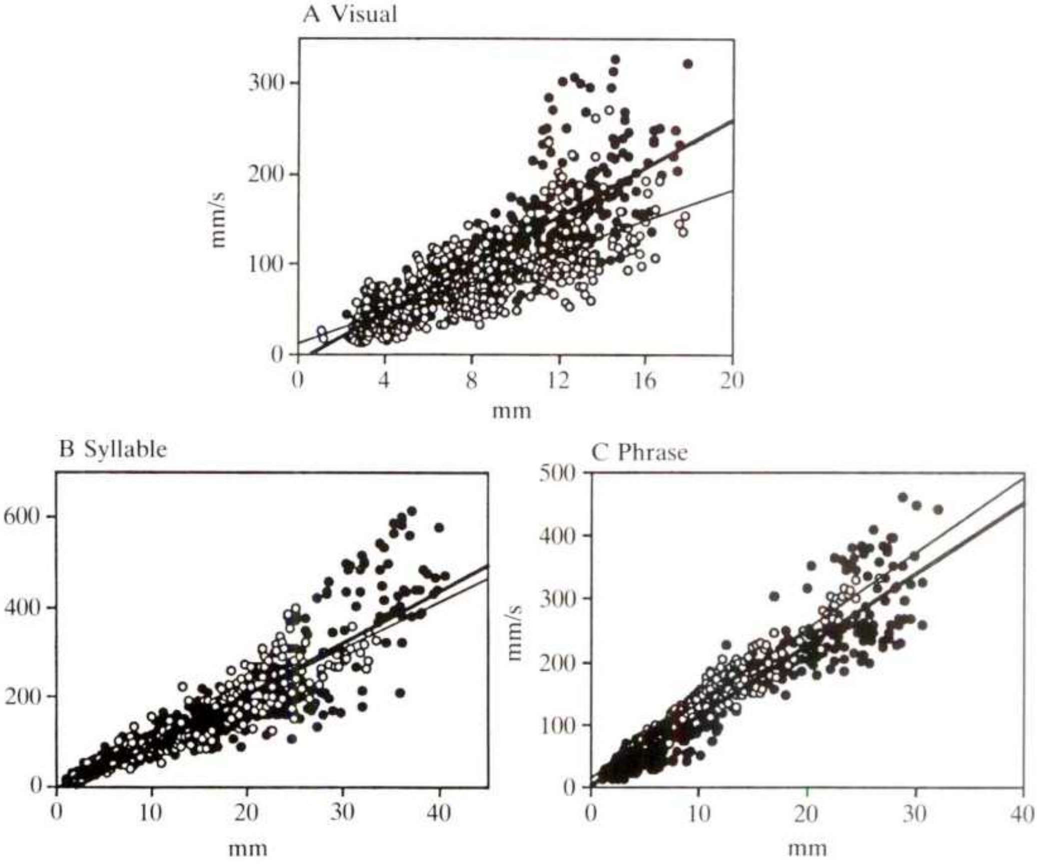

FIG. 3. Peak velocity by amplitude for visually-guided movements (A), a single syllable (B) and 'da' within a speech phrase (C). All data for PD (open circles) and normal (closed circles) subjects are represented. Thick lines represent line of best fit for the normal group, while thin lines are the PD line of best fit. Note: ordinates and abscissae were selected to optimize data presentation and are not consistent across plots.

were significantly shorter than normal ($F_{group} = 13.05$, df(1,25), $P = 0.001$; $F_{amplitude} = $ n.s., $F_{group \times amplitude} = $ n.s.), with no PD vs control differences evidenced for the isolated syllable condition.

As with previous reports, PD subjects manifested significant reductions in the ratio of peak velocity to movement amplitude during visually-guided movements ($F_{group} = 5.42$, df(1,30), $P = 0.027$; $F_{amplitude} = $ n.s., $F_{group \times amplitude} = $ n.s.). However, PD subjects had significantly greater ratios than normal for movements embedded within speech phrases ($F_{group} = 8.20$, df(1,25), $P = 0.008$; $F_{amplitude} = $ n.s., $F_{group \times amplitude} = $ n.s.) and were not different from the control group for movements within the isolated syllable. In line with the above observations, absolute peak velocity magnitudes were different between groups only for visually-guided movements, during which PD subjects were significantly slower ($F_{group} = 6.82$, df(1,30), $P = 0.01$).

Movement accuracy. Although the difference was not statistically significant, PD subjects tended to achieve visual target positions more consistently than the normal group (fig. 4); the normal subjects manifested overshoot significantly more often ($F = 5.65$, df(1,30), $P \leq 0.025$). While, as noted, percentage error in endpoint position relative to the target location was not significantly different between groups, it was significantly greater for 4 mm targets than for 8 or 12 mm targets. Because spatial target error for speech could not be evaluated for specified position, coefficients of variation were calculated for movement amplitude data as an estimate of movement accuracy. A three-factor ANOVA (group, task, movement amplitude) indicated only a significant main effect for 'amplitude' ($F_{amplitude} = 14.51$, $P \leq 0.001$). Post hoc least significant differences revealed significantly larger variances for the 'small' movements ($P < 0.05$), relative to 'medium' and 'large' ones. For all tasks in this study it was thus clear that PD and normal groups did not evidence significant differences in movement accuracy, nor was performance of any task more accurate than another. However, for all subjects

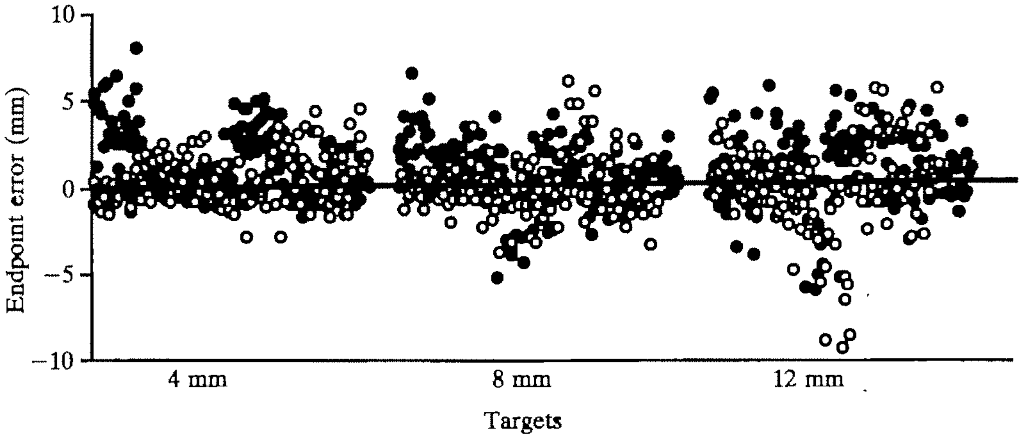

FIG. 4. Endpoint position error for all visual task trials from all normal (filled circles) and parkinsonian (open circles) subjects. Zero position on the ordinate reflects target position, positive values indicate target overshoot, and negative values indicate endpoint undershoot.

larger movements were produced more 'accurately' than smaller ones, at least on a relative basis. Subjectively, all PD and normal subjects reported greater ease in reaching the 8 or 12 mm versus the 4 mm targets.

Velocity profile analysis

All speech movements in both groups were characterized by single symmetric velocity profiles. Illustrating this point are phrase task trials from 1 PD subject in fig. 5A. In contrast, as illustrated in fig. 5B, there were often a number of velocity peaks during visually-guided PD movements. With visual targets, PD subjects manifested a significantly greater proportion of trials with 3 or more velocity peaks (F = 4.23, $P \leq 0.05$), while normal subjects tended to complete movements with smooth single-peaked velocity profiles (fig. 6). The number of velocity peaks within a movement trial was positively correlated with movement time to target in both groups (PD: r = 0.846; controls: r = 0.750).

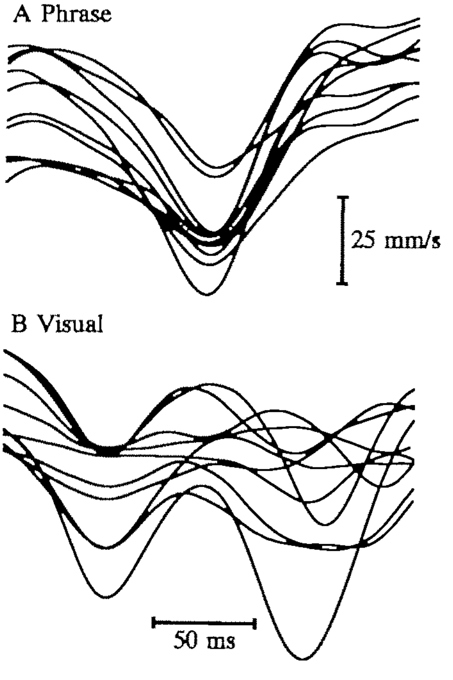

FIG. 5. A, single-peaked velocity profiles were found in jaw lowering movements within speech phrases. Data shown here are 10 trials from a representative PD subject (*Case 4*). B, in contrast, multiple velocity peaks were often found during visually-guided movements. Ten such trials are shown from a PD subject (*Case 4*)

DISCUSSION

The present study offers insight into the degree to which task parameters, such as familiarity, serial execution and visual guidance influence PD motor function within a single muscle group. These task-dependent variations were apparent on a number of measures.

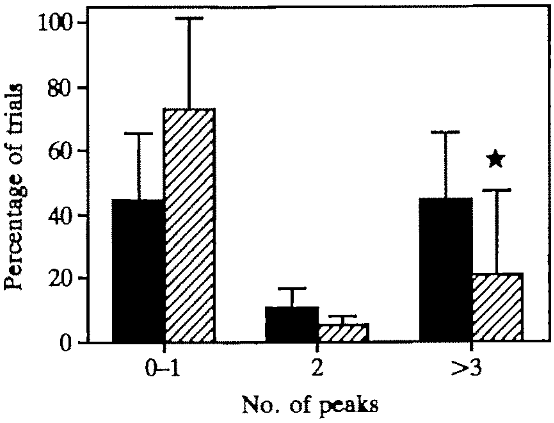

FIG. 6. PD subjects (filled bars) manifested three or more velocity peaks within a visually-guided movement trial significantly more often (star) than normal controls (hatched bars). In contrast, normal controls manifested single peaks more often, although not at statistically significant levels.

Peak velocity-amplitude

PD jaw movements were characterized by reductions in velocity/amplitude ratios only when performed under visual guidance; there was no such impairment during speech actions. Although direct statistical analyses were confined to individual tasks, this observation clearly suggests that velocity saturation is not a pervasive characteristic of PD, but occurs primarily in certain, perhaps novel, or unnatural contexts. Indeed the present study suggested a continuum whereby the most unfamiliar and unnatural movements (namely visually-guided jaw movements) were performed with lowest peak velocities, semifamiliar isolated speech syllables were characterized by intermediate velocities, and speech movements generated within natural sequential contexts had greater than normal levels. Such findings suggest that the degree of expression of PD motor abnormalities is highly related to the task in hand.

The patterns of task-dependent alterations in velocity-amplitude ratios observed in this study may help explain certain inconsistencies found in motor literature on Parkinson's disease. For example, while reports of velocity-amplitude reductions during visually-guided PD arm movements are frequent (Draper and Johns, 1964; Flowers, 1975, 1976, 1978; Hallett and Khoshbin, 1980; Accornero *et al.*, 1985; Berardelli *et al.*, 1986), Teasdale and Stelmach (1988) found normal adjustments in limb movement times when accuracy requirements were minimized. Similarly, normal movement time and peak velocity during sequential movements for speech have been reported (Connor *et al.*, 1989; Forrest *et al.*, 1989), but because these experiments involved different muscle groups for a variety of novel and natural contexts, factors accounting for these task-dependent alterations could not be determined. In this respect, the current work provides a more systematic approach toward the identification and quantification of such factors.

The results of previous studies would suggest greater deficits in PD motor performance for larger or sequential movements (Hallett and Khoshbin, 1980; Benecke *et al.*, 1987).

This was not the case in the present study of orofacial movements in which movements within a sequential speech context manifested the fastest speeds and movements with the large amplitudes were relatively more accurate.

Movement accuracy

Movement accuracy was not reduced relative to normal in PD subjects during movements towards a visual target or when accuracy was defined as the degree of amplitude variation across 30 speech movement trials. This finding of spatially accurate, but slow, PD movements has been reported previously (Berardelli *et al.*, 1986; *see also* Accornero *et al.*, 1985; Teasdale and Stelmach, 1988). However, this is not a consistent finding in that reductions in spatial accuracy (compared with normal) have also been reported, particularly when visual feedback is not provided (Viallet *et al.*, 1987; Zeffiro and Claman, 1988). These discrepancies could again be due to task differences or differential degrees and kinds of impairment among limb and orofacial muscle groups.

Velocity profiles

While reports of smooth, symmetric single-peaked velocity profiles during normal limb movements are commonplace (Morasso, 1981; Abend *et al.*, 1982; Atkeson and Hollerbach, 1985), PD subjects reportedly use several velocity peaks or agonist-antagonist muscle bursts to reach a limb movement amplitude goal (Draper and Johns, 1964; Hallett and Khoshbin, 1980; R. Inzelberg *et al.*, unpublished). This implies that PD subjects are unable to generate a single adequate burst of muscle force to produce the desired movement amplitude and must produce a series of segmented contractions or successive approximations toward the target. In agreement with these previously reported results, this study also found segmented movements (e.g., multiple velocity peaks) during visually-guided jaw opening more often in PD subjects compared with normal controls. However, task-related effects also were observed on this measure. As found previously (N. P. Connor *et al.*, 1989; unpublished observations), single-peaked velocity profiles were always present for speech tasks in both groups. Therefore the segmented or jerky movements observed in PD also appear to be task-dependent and seemingly more likely to occur during tasks with externally applied visual targets.

Neural substrates

Differential impairment of limb and orofacial muscle groups in PD, or differences with task, may underlie disparities among results of the present study and those found in the limb movement literature. Neural substrates for this differential impairment may include segregation of 'leg', 'arm' and 'face' areas within the putamen and globus pallidus (*see* Crutcher and DeLong, 1984; DeLong and Alexander, 1987). If one anatomical region is more impaired than another, differential motor impairments would be expected when movements from several body parts are compared. Interestingly, groupings of putamen neurons with similar response properties and clusters of cells evoking only certain types of movements from specific body parts have been identified within these larger anatomically segregated regions (Crutcher and DeLong, 1984; Alexander and DeLong, 1985*a*, *b*). Such functional cell groupings may account for task-dependent movement effects within an anatomical region, as observed in the present study.

Consideration of basal ganglia connectivity may also explain apparent sensorimotor impairments related to visual guidance in PD subjects (*see* Schneider, 1984; Schneider *et al.*, 1986). Because the basal ganglia receive inputs from virtually all cortical areas (*see* DeLong and Georgopoulos, 1981), disruptions in sensory to motor linkages may underlie the observed deficits in PD motor performance (Schneider, 1987). Lesion studies in rats, cats and awake primates suggest that the basal ganglia may have a primary role in sensorimotor integration (*see* Aldridge *et al.*, 1980; Schneider, 1984, 1987). For example, basal ganglia lesions have resulted in severe visuomotor disruptions, which were often characterized by PD-like restrictions in motor initiation (Denny-Brown and Yanagisawa, 1976; Pisa, 1988). In some lesion experiments, deficits were observed only in tasks requiring somatosensory or visual feedback control, while familiar motor tasks remained unaffected (*see* Labuszewski *et al.*, 1981; Schneider, 1987). Recordings of single neurons in caudate, putamen and globus pallidus have also revealed some context-dependent neuronal firing such that cells responsive to a visuomotor cue in one condition do not fire in alternative experimental situations (Aldridge *et al.*, 1980; Hikosaka and Wurtz, 1983; Alexander, 1987; Rolls and Williams, 1987). Therefore in paradigms using a visual stimulus or novel tasks, the quantitative evidence regarding PD poverty of movement and delayed response time may be a function of the integratory role of the basal ganglia rather than a motor or sensory impairment per se (Schneider, 1987).

Overall, however, the role of visual guidance in PD motor control has been difficult to establish. Although some work has suggested an over-reliance on visual input by PD subjects during movement (Flowers, 1975, 1976, 1978), other reports have indicated improvement in movement speed when visual feedback was removed (R. Inzelberg *et al.*, unpublished; Zeffiro and Claman, 1988). Clinical reports add to the confusion; in some contexts the presence of a visual stimulus can aid movement initiation, while in others it causes a 'freezing' response (Schneider, 1987). Regardless of these confusions, results of performance alterations in PD subjects plus/minus visual input are consistent with the view of basal ganglia projections as primary pathways for the modulation of sensorimotor information flow (Schneider, 1987). Further behavioural experiments in which sensory and motor variables are carefully manipulated may provide an indication of the neurophysiological mechanisms underlying task-specific motor disruptions in PD.

Conclusions

Speech motor functions appear to be model systems for the investigation and characterization of PD motor impairments since these are sequential movements which occur naturally without visual guidance. Indeed this investigation has shown that the classical descriptors of PD cannot be applied to all movement tasks within a given patient. It may not be possible to characterize functional and natural motor deficits of daily living by examining visually-guided, single joint movements of the upper extremity.

Obviously, as recently suggested, future studies of motor control in PD also must consider the movement context, since task parameters greatly influence the movement's organization and execution (*see* Teasdale and Stelmach, 1988). To this end, the present study suggests that natural, familiar movements, without externally-imposed spatial targets, are performed at or above normal speed by PD subjects. Therefore in attempting to characterize motor disruptions in PD as they occur in everyday life, it is critical to determine which movement contexts, task parameters, or sensorimotor interactions influence motor performance.

ACKNOWLEDGEMENTS

The authors are grateful for the assistance of the following individuals: Drs Miroslav Backonja and Roxanne DePaul for clinical examinations, and for technical assistance, Geri Torti and Gregory Turner. This work was supported by National Institutes of Health Grant NS-13274.

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(*Received October 10, 1989. Revised January 16, 1990. Accepted March 20, 1990*)

Brain (1991), **114**, 333–348

EFFECTS OF ANTICONVULSANT TREATMENT AND LOW LEVELS OF FOLATE AND THIAMINE ON AMINE METABOLITES IN CEREBROSPINAL FLUID

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SUMMARY

A total of 157 epileptic patients were studied with respect to (1) biogenic amine precursors and metabolites in the CSF, (2) levels of folate and thiamine in the blood and CSF, (3) length of treatment with phenytoin (PHT), (4) PHT intoxication, (5) CNS atrophy. Alterations in CSF amine metabolite levels were related primarily to PHT intoxication, and low CSF folate and thiamine levels, but not to length of treatment or CNS atrophy. PHT intoxication increased CSF 5-hydroxyindoleacetic acid (5HIAA). Low folate levels were associated with decreased CSF 5HIAA and homovanillic acid, while low thiamine levels were associated with decreased CSF 5HIAA and 3-methyoxy-4-hydroxyphenylethylene glycol. It remains to be seen to what extent these alterations in biogenic amine metabolism, mediated by low CNS vitamin levels, also lead to deficits in cerebral function.

INTRODUCTION

Measurement of biogenic amine metabolites in the cerebrospinal fluid (CSF) of epileptic patients has produced a variety of apparently contradictory results. Shaywitz *et al.* (1975) found that CSF concentration of 5-hydroxyindoleacetic acid (5HIAA) and homovanillic acid (HVA) were low in epileptic children who were being treated with a variety of drugs, including phenobarbital and phenytoin (PHT), relative to the levels in children with other disorders. Chadwick *et al.* (1977) measured CSF 5HIAA and HVA in adult epileptics who were either untreated or being treated with a variety of anticonvulsants including phenobarbital and PHT, and in adult neurological controls. No changes were seen in the untreated patients but anticonvulsant treatment increased 5HIAA but not HVA. The rise in CSF 5HIAA was related to the number of anticonvulsants administered and the serum levels of phenobarbital and PHT. Particularly high values were seen in anticonvulsant-intoxicated patients. The results of Young *et al.* (1980) in adult epileptics undergoing pneumoencephalography were similar to those of Shaywitz *et al.* (1975). There was no difference in CSF 5HIAA levels between untreated epileptics and neurological controls, but epileptics who were being treated with one or more anticonvulsants, including phenobarbital and PHT, had significantly lower mean lumbar CSF 5HIAA than untreated epileptics. Laxer *et al.* (1979) studied similar groups of patients and found no difference between CSF 5HIAA, HVA and 3-methoxy-4-

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hydroxyphenylethylene glycol (MHPG) in treated and untreated epileptics. However, the groups were relatively small and there was a trend to lower 5HIAA in the treated epileptics. Finally, Habel *et al.* (1981) measured CSF 5HIAA and HVA in untreated children with febrile convulsions, afebrile convulsions, febrile controls and afebrile controls. There were no significant differences between groups. The data above suggest that convulsions do not influence CSF amine metabolite levels but the effect of anticonvulsant drugs is not clear as increases, decreases and no change in CSF 5HIAA have all been reported.

PHT is one of several anticonvulsants known to decrease intestinal absorption of folic acid (Elsborg, 1974). This can lead to folate deficiency. We have shown that dietary folate deficiency can result in a lowering of rat brain 5-hydroxytryptamine (5HT) (Botez *et al.*, 1979). In patients with folate deficiency due to inadequate intake or malabsorption, CSF 5HIAA was low when folate-responsive neuropsychiatric symptoms occurred. In these patients folate treatment increased CSF 5HIAA to normal levels (Botez *et al.*, 1982*b*). In a preliminary study, a lowering of blood and CSF thiamine levels was found in some epileptics treated chronically with PHT (Botez *et al.*, 1982*a*). Studies in the rat have shown that thiamine deficiency can cause degeneration of 5HT neurons in the cerebellum (Plaitakis *et al.*, 1978). We found low CSF 5HIAA in 3 out of 5 patients with low CSF thiamine. In all 3, CSF 5HIAA returned to normal after thiamine supplementation (Botez *et al.*, 1982*c*). These data raise the possibility that the low CSF 5HIAA levels in treated epileptics may be due, in some patients, to deficiencies in folate and/or thiamine.

The purpose of the present study was to determine how PHT intoxication, and low levels of folate or thiamine, influence CSF amine metabolite levels in epileptics undergoing treatment with PHT. We also looked at the possible roles of length of treatment and CNS atrophy as factors influencing the biochemical measures.

PATIENTS AND METHODS

Patients

Our inclusion and exclusion criteria are similar to those used by Mattson *et al.* (1983) in their study on the efficacy and toxicity of antiepileptic drugs. The inclusion criteria were (1) men and women aged 18 yrs and over attending epilepsy outpatient clinics; (2) patients diagnosed as chronic or de novo idiopathic epileptics with type of seizures classified according to the international classification (Commission on Classification and Terminology of the International League Against Epilepsy, 1981). The exclusion criteria were (1) patients with epileptic seizures due to a tumour in evolution or with degenerative, metabolic or demyelinating disease or acute infection; (2) epileptics who ingested an average of more than 2 alcoholic drinks a day; (3) psychotic patients and those with severe depression; (4) patients taking narcotics; (5) patients with epileptic seizures due to a medical disease in evolution; (6) patients taking other medication that could possibly interfere with the metabolism or absorption of folate or thiamine; (7) all long-term institutionalized patients; (8) pregnant women; and (9) evidence of frank dietary deficiencies from a routine dietary assessment. Epileptic patients showing clinical and biological signs of PHT intoxication (vestibulocerebellar syndrome plus serum PHT above 20 μg/ml) were immediately admitted to hospital and included in the protocol if the exclusion criteria allowed.

In our study, de novo and PHT-treated or PHT and phenobarbital-treated epileptics were included; epileptics taking other medications were excluded from the study. Of the PHT-treated patients, 35% also received phenobarbital. Epileptic patients underwent CT scan studies before being hospitalized. All patients were hospitalized at the Metabolic Ward of Hôtel Dieu Hospital, Montreal during a 5 yr period. Both de novo and chronic epileptics were admitted sequentially in the order of presentation at the outpatient clinics. The patients gave informed consent. The protocol was approved by the ethics committee of the Hospital.

A total of 157 epileptics (de novo patients and chronic epileptics) were included in this study; there were 68 men and 89 women. In this paper, we used two classifications, i.e., patients with tonic-clonic generalized seizures and patients with complex partial seizures evolving to tonic-clonic generalized seizures. Progression to tonic-clonic generalized seizures had occurred in all patients with complex partial seizures, but did not necessarily occur with each seizure. Tables 1 and 2 give the number of patients, the types of seizures and the EEG interictal expression.

TABLE 1 CLINICAL AND EEG DATA IN PATIENTS WITH GENERALIZED SEIZURES

	*Number and sex of patients**	
	Treated epileptics	*De novo epileptics*
Type of seizure		
Tonic-clonic	35 (78%). 20M,15F	12 (75%) 7M,5F
Clonic	4 (9%) 2M,2F	2 (13%) 2M
Tonic	6 (13%) 5M,1F	2 (13%) 1M,1F
EEG interictal expression		
3 Hz and slow wave discharges (classical complex)	16 (36%): 11M,5F	6 (38%) 4M,2F
Polyspike and slow wave complexes	10 (22%): 7M,3F	3 (19%). 1M,2F
Sharp and slow wave discharges sometimes asymmetric	10 (22%): 4M,6F	5 (31%): 4M,1F
Normal EEG	7 (16%) 3M,4F	2 (13%) 1M,1F
Temporal slowing	2 (4%) 2M	0

* Numbers are given with the percentage of the total samples and the numbers of males (M) and females (F). The EEGs were carried out with a Grass 16-channel apparatus. EEGs were performed at rest and after different activation procedures, i e., spontaneous or induced sleep, hyperventilation and photic stimulation

Neuroradiological assessment

The neuroradiological investigation was carried out by Dr J. L. Vezina with an EMI-5005 CT scan apparatus and consisted of 10 mm axial sections of the brain and 5 mm high resolution sections of the posterior fossa. Cerebral cortical atrophy was assessed according to the criteria of Huckman *et al.* (1975) and of Hahn and Rim (1976). Cerebellar and brainstem atrophy was evaluated according to the criteria of Koller *et al.* (1981), namely the cerebellar hemispheres were considered atrophied when folia were visible, whereas vermian atrophy was considered present on finding 2 or more sulci in the midline As suggested by Koller *et al.* (1981), the superior cerebellar, cerebellopontine angle, quadrigeminal and lateral basal cisterns were evaluated subjectively along with the cisterna magna and the fourth ventricle. The assessments were done independently by 2 observers (Drs J. L. Vezina and E. Attig) without knowledge of the clinical data retained by the neurologist Details regarding the neuroradiological ratios in patients with cerebellar atrophy and those with normal CT scans are discussed elsewhere (Attig, 1985; Botez *et al.*, 1988).

Clinical measures

Physical examination carried out by 2 neurologists, included grading of ataxia (tested on heel-to-toe walking), slurring of speech, alternate tapping of the hands, finger-nose test and gaze paretic nystagmus. Grades were 0 (normal), 1 (slight), 2 (moderate) or 3 (severe abnormality). The length of the illness was

TABLE 2. CLINICAL AND EEG DATA IN PATIENTS WITH COMPLEX PARTIAL SEIZURES WHICH SOMETIMES EVOLVED TO GENERALIZED TONIC-CLONIC SEIZURES

	*Number and sex of patients**	
	Treated epileptics	*De novo epileptics*
Type of seizure		
With motor signs	13 (17%): 5M,8F	3 (16%): 2M,1F
With somatosensory or sensory symptoms	11 (14%): 4M,7F	4 (21%): 2M,2F
With autonomic symptoms	8 (10%): 2M,6F	1 (5%): 1F
With psychic symptoms	28 (36%): 9M,19F	8 (42%): 1M,7F
With automatisms	17 (22%): 6M,11F	3 (16%): 3F
EEG interictal expression (localization of epileptic focus)		
Anterior midtemporal	22 (29%): 10M,12F	7 (37%): 1M,6F
Medial temporal	11 (14%): 3M,8F	3 (16%): 1M,2F
Posterior temporal	6 (8%): 2M,4F	2 (11%): 1M,1F
Occipital	3 (4%): 2M,1F	0
Unilateral frontal	11 (15%): 2M,9F	2 (11%): 1M,1F
Bilateral generally asynchronous focus	16 (21%): 5M,11F	2 (11%): 2F
Normal EEG	8 (10%): 2M,6F	3 (16%): 1M,2F

* Information in the footnote to Table 1 applies to Table 2.

calculated in months for each patient; the number of grand mal and other seizures was estimated for the entire lifetime of the patient (de Bittencourt, 1983) on the basis of clinical records from our hospital and from Montreal Children's and Sainte Justine Hospitals. The total amount of PHT ingested during the patient's lifetime was calculated retrospectively (in g) from the same records.

Biochemical measures

CSF was collected by lumbar puncture; the procedure was done on fasting patients between 8 a.m. and 9.30 a.m. All patients underwent routine laboratory tests and serum and CSF PHT determinations. Therapeutic serum PHT values are from 10 to 20 μg/ml; the CSF PHT content is usually 10% of the serum value (Laxer *et al.*, 1979). PHT intoxication was defined either on the basis of plasma PHT levels of more than 20 μg/ml and the presence of acute cerebellar ataxia, or on the basis of PHT levels of more than 20 μg/ml with occasional symptoms of intoxication such as dizziness and equilibrium disturbances.

Serum and CSF folate levels were determined using *Lactobacillus casei* ATCC 7649 (Herbert, 1966; Wells and Casey, 1967). Because we were interested in effects on the CNS, low folate levels were defined by CSF rather than serum levels. We defined low folate levels as values of CSF folate less than 19 ng/ml, borderline values were between 19 and 23 ng/ml, while normal values were those above 23 ng/ml. These divisions were defined partly on the basis of control CSF values obtained using the same assay. Control values for CSF folate were obtained from patients with lumbar disc herniations who underwent myelograms during a brief stay in hospital. Patients were excluded if they had a medical or neurological disease in evolution, if they had taken any drugs or vitamins in the previous 2 wks, or if a dietary assessment revealed an inadequate diet. In the controls (50 subjects of whom 23 were men and 27 women, with a mean age ± SD of 46.6 ± 13.2 yrs) the CSF folate levels were (mean ± SD) 39.9 ± 12.9 ng/ml. The lower limit was 18.9 ng/ml.

Using the microbiological assay normal mean CSF folate levels were reported in different studies as 31 ng/ml (Herbert and Zalusky 1961), 23.6 ng/ml (Wells and Casey, 1967), 20.9 ng/ml (Reynolds *et al.*, 1969), 31.6 ng/ml (Reynolds *et al.*, 1972), 26.4 ng/ml (Streiff *et al.*, 1972), 26.3 ng/ml (Reizenstein and Lund, 1973) and 31 ng/ml (Waxman, 1979). The mean ±SD of serum folate values for the low folate and borderline groups in the present study were [illegible]78 ± 2.48 ng/ml and 4.59 ± 1.78 ng/ml. As we have pointed out in a recent review, the serum folate [illegible] used to define folate deficiency is a matter of controversy. In different studies the values used have [illegible] from 2 to 8 ng/ml (Young and Ghadirian, 1989). The limits used in this study were designed to exclude any symptoms of deficiency such as mild megaloblastic anaemia in the control group rather than to ensure that all patients in the low folate group were exhibiting symptoms. Thus, our low folate group is not described as a folate deficient group, as was done in the other studies mentioned above. Nonetheless, the fact that we found biochemical differences between subjects in the ranges which we defined as normal and low folate (Table 4) suggests that our definition has some functional utility.

The whole blood thiamine levels were assayed by the microbiological method in duplicate, using *Lactobacillus fermenti* (Baker and Frank, 1968). When low values of thiamine were found, an additional incubation was carried out with added thiamine to check that no inhibitors of bacterial growth were present (Bachevalier *et al.*, 1981). Using this method of verification, not used previously, the normal values in our laboratory are higher than 40 ng/ml for blood (mean ±SD was 50.3 ± 8.9) and higher than 24 ng/ml for CSF thiamine levels (mean ±SD was 33.9 ± 6.7). Normal thiamine values, like the normal folate levels, were established in 56 control subjects (30 males and 26 females) who had a normal dietary assessment, were not alcoholics and were not suffering from any kind of medical disease in evolution. Blood and CSF samples were taken during myelograms for lumbar disc herniations in the Radiology Department of Hôtel Dieu Hospital. Our normal CSF thiamine levels are in good agreement with those reported by Baker and Frank (1968) and by Rindi *et al.* (1981). Borderline CSF thiamine values were considered those between 18.6 and 23.9 ng/ml, whereas low thiamine levels were defined as CSF values less than 18.5 ng/ml.

For the purpose of classifications described above, vitamin status was determined solely on the basis of CSF vitamin levels without taking into account the plasma levels. This was done because the important variable is the amount of vitamin available to the brain. Also, low plasma levels due to borderline deficiencies might be corrected by improved nutrition during a short stay in hospital, but recovery of CSF levels would take longer.

Tryptophan, tyrosine, 5HIAA, HVA and MHPG were measured in the CSF using high performance liquid chromatography with fluorometric and electrochemical detection (Anderson *et al.*, 1979, 1981). All of the biochemical analyses were done blind to the clinical assessment.

Among the 122 treated epileptics who were studied, 43 (35%) received both phenobarbitone and PHT, while the remainder received only PHT. Phenobarbitone treatment of epileptics can sometimes induce a megaloblastic anaemia that is responsive to folate (Chanarin *et al.*, 1960). A preliminary statistical analysis was therefore carried out to see if the patients who received PHT alone differed from those who received PHT and phenobarbitone on any of the measures studied.

For the main analysis the data set was broken down into categories using different classification schemes based on presence or absence of anticonvulsant-induced intoxication, low CSF folate levels, low CSF thiamine levels, CNS atrophy, and the length of PHT treatment. For each classification, we first examined the distribution of the various parameters, in order to detect outliers or problems related to homogeneity of variance. We then defined, for each classification, k-1 comparisons that were of particular interest, k being the number of groups defined by the classification (for example, if 3 groups were defined by the classification, 2 comparisons were planned). This procedure led us to define a priori the following comparisons. In classification 1 (Table 3) values of PHT intoxicated patients were compared with those of patients well controlled by the medication (contrast 1) and with those of untreated patients (contrast 2). In classifications 2 (Table 4) and 3 (Table 5) patients with normal levels of folate or thiamine in their CSF were compared with those with low vitamin levels (contrast 1), and with those with borderline normal values (contrast 2). In classification 4 (Table 6) the parameters of patients receiving no treatment were compared with those of patients treated less than 5 yrs (contrast 1) and with those of patients treated for more than 5 yrs (contrast 2). In classification 5 (Table 7) the parameters in patients with no brain atrophy were compared with those of patients with exclusively cerebellar atrophy (contrast 1) and with those with both cerebellar and cerebral atrophy (contrast 2). The analyses of the 5 different classifications were done independently of one another. After the comparisons had been defined, we performed a one-way analysis of variance on each of the classifications, in order to test these classifications on an a priori basis. Because the number of comparisons was a priori limited, this procedure allowed us to set the alpha level of 0.05 (Kirk, 1968).

RESULTS

Only 5 patients showed signs of cerebellar ataxia (2, slight; 2, moderate; 1, severe); 17 other patients were defined as being intoxicated with PHT at the time of clinical examination and 21 others were defined as having had PHT intoxication at some previous time on the basis of plasma PHT levels above 20 μg/ml together with occasional symptoms of intoxication such as dizziness and equilibrium disturbances. All other patients had normal clinical examinations.

A comparison of the various measures in patients receiving phenobarbitone plus PHT (n = 43) against those receiving PHT alone (n = 79) revealed no significant differences. For example, CSF folate and thiamine values in the two groups were (mean ±SD in ng/ml) 21.8 ± 10.0 vs 24.4 ± 14.5 and 23.5 ± 9.0 vs 23.8 ± 8.0. No significant differences were found between the two groups for age, sex, number of tonic-clonic and complex partial seizures, blood vitamin levels and CSF amine precursor and metabolite levels. There was a tendency for CSF HVA to be higher in patients treated with PHT plus phenobarbitone (50.2 ± 29.2 vs 41.5 ± 21.2 ng/ml, $P < 0.07$).

Table 3 shows some of the factors related to PHT intoxication. As might be expected mean blood and CSF PHT levels are at least twice as high in intoxicated patients as in those who were not intoxicated. The main finding of interest is that CSF 5HIAA is elevated in intoxicated patients. This finding is not due to elevated tryptophan levels, which are not increased in intoxicated patients, nor is it due to the fact that the intoxicated patients are significantly older than patients who are not intoxicated. Although CSF 5HIAA increases with age, the increase is relatively small (Young *et al.*, 1980). The age difference between intoxicated and nonintoxicated subjects is not large enough to account for the one-third increase in CSF 5HIAA in intoxicated patients. Low vitamin levels play no role in intoxication. In intoxicated patients folate levels are significantly lower than in untreated patients. However, the mean values for serum and CSF folate in the intoxicated subjects are well above levels we defined as low, and are not significantly different from levels found in patients who were not intoxicated. The increase in CSF 5HIAA is also not due to past intoxication. Mean values for CSF 5HIAA are similar in the groups who either had or had not been intoxicated in the past but were well controlled at the time of the study.

Factors associated with low CSF folate levels are shown in Table 4. One-third of the patients studied showed low folate levels and another 9% had borderline folate levels. Patients with low folate did not differ from those with normal folate on age, but had significantly higher lifetime PHT dosage, blood and CSF PHT levels and duration of epilepsy. This is not surprising as PHT tends to inhibit absorption of folate (Rosenberg *et al.* 1979). Higher levels given for a longer time will do this more effectively. Patients who had low CSF folate had significantly lower CSF thiamine. The mean CSF thiamine for patients with low CSF folate was in the borderline range. As borderline thiamine levels are not associated with a decline in CSF 5HIAA (Table 5), the low levels of CSF 5HIAA found in patients with low folate were presumably due to low folate levels and not to a decline in thiamine levels. In addition to a lowering of CSF 5HIAA, low folate was associated with low CSF HVA levels. Values for the borderline folate subjects in general showed similar trends to those for low folate subjects. However, most of the differences between normal and borderline subjects were smaller than those between

TABLE 3. FACTORS ASSOCIATED WITH PHT INTOXICATION*

	Variable				*P values*	
	Group 1 *Intoxicated*	*Group 2a* *Well controlled but past intoxication*	*Group 2b* *Well controlled*	*Group 3* *No treatment*	*1 vs 2*	*1 vs 3*
Age (yrs)	51±19(21)	40±14(21)	46±14(80)	42±16(35)	<0.05	<0.025
Lifetime PHT (g)	1750±1500(21)	1520±1190(21)	1660±1180(80)		n.s.	
Serum PHT (μg/ml)	25.0±9.1(21)	13.4±3.3(21)	11.4±3.3(79)		<0.001	
CSF PHT (μg/ml)	3.76±2.47(21)	1.76±0.90(21)	1.78±1.10(79)		<0.001	
Serum folate (ng/ml)	6.26±4.93(19)	3.55±1.78(17)	5.94±3.60(75)	9.00±3.71(28)	n.s.	<0.05
CSF folate (ng/ml)	26.1±10.0(19)	20.0±15.7(17)	23.7±13.3(74)	36.4±15.2(29)	n.s.	<0.025
Blood thiamine (ng/ml)	44.8±10.9(20)	46.9±9.3(20)	45.5±12.2(78)	46.6±9.7(35)	n.s.	n.s.
CSF thiamine (ng/ml)	23.5±7.2(21)	23.2±10.1(20)	23.9±8.3(75)	27.0±5.9(35)	n.s.	<0.1
CSF tryptophan (ng/ml)	311±118(21)	347±118(21)	345±91(78)	317±119(34)	n.s.	n.s.
CSF tyrosine (ng/ml)	2080±1490(21)	2040±1690(21)	2360±1930(76)	1460±550(34)	n.s.	<0.1
CSF 5HIAA (ng/ml)	28.6±23.9(21)	21.3±11.9(21)	20.2±10.0(78)	20.4±14.6(35)	<0.05	<0.05
CSF HVA (ng/ml)	46.1±29.0(21)	43.9±24.8(21)	41.9±23.6(75)	35.2±18.7(34)	n.s.	<0.1
CSF MHPG (ng/ml)	9.27±3.38(21)	11.79±8.33(21)	8.86±6.13(79)	9.50±6.60(35)	n.s.	n.s.

* All values are given as mean±SD (no. of patients)

TABLE 4 FACTORS ASSOCIATED WITH VARYING CSF FOLATE LEVELS*

	Variable			*P values*	
	Group 1 *Low folate*	*Group 2* *Borderline*	*Group 3* *Normal folate*	*1 vs 3*	*2 vs 3*
Age (yrs)	44±14(49)	40±14(13)	46±16(81)	n.s.	n.s.
Lifetime PHT (g)	1810±1420(49)	894±971(13)	1010±1140(81)	<0.001	n.s.
Serum PHT (μg/ml)	13.2±6.65(48)	11.2±8.3(13)	9.71±8.98(80)	<0.05	n.s.
CSF PHT (μg/ml)	2.08±1.74(48)	2.12±2.66(13)	1.40±1.39(79)	<0.05	n.s.
Duration of epilepsy (mos)	233±157(49)	107±99(13)	166±172(81)	<0.05	n.s.
No. of grand mal seizures	30.5±46.6(49)	24.0±49.2(13)	17.3±32.1(80)	<0.1	n.s.
No. of complex partial seizures	208±418(49)	158±291(13)	306±1030(81)	n.s.	n.s.
Serum folate (ng/ml)	3.78±2.48(48)	4.59±1.78(13)	8.26±3.98(76)	<0.001	<0.001
CSF folate (ng/ml)	12.3±5.1(48)	21.0±0.8(13)	35.9±12.0(77)		
Blood thiamine (ng/ml)	44.1±10.2(48)	42.6±9.4(12)	46.3±118(80)	n.s.	n.s.
CSF thiamine (ng/ml)	22.3±8.1(47)	24.1±12.0(12)	25.5±7.1(79)	<0.05	n.s.
CSF tryptophan (ng/ml)	343±106(48)	349±56(13)	316±103(80)	n.s.	n.s
CSF tyrosine (ng/ml)	2220±1690(47)	1600±670(13)	2120±1850(78)	n.s.	<0.1
CSF 5HIAA (ng/ml)	17.7±6.6(48)	19.2±5.8(13)	23.1±17.8(81)	<0.05	n.s.
CSF HVA (ng/ml)	35.2±16.8(47)	43.8±21.3(13)	44.8±27.2(78)	<0.05	n.s.
CSF MHPG (ng/ml)	8.23±4.87(49)	10.05±7.32(13)	9.14±5.93(81)	n.s.	n.s.

* All values are given as mean ± SD (no. of patients).

normal and low folate subjects. In view of this, and the small number of subjects in the borderline group, it is impossible to say whether the lack of any statistical differences between borderline and normal subjects reflects a lack of any real difference.

Approximately one-quarter of the patients had low CSF thiamine and a further quarter had borderline thiamine levels (Table 5). Low thiamine levels were associated with higher lifetime PHT and higher CSF PHT, but the differences from the group with normal thiamine levels did not achieve statistical significance. In the patients with low thiamine, CSF thiamine levels were about half the level found in patients with normal thiamine, but only a 10% decline was seen in blood levels. As the samples of blood and CSF were taken several days after the patients were admitted to hospital, the hospital diet may have helped to replete low blood thiamine levels. Presumably longer repletion would be necessary to see substantial changes in CSF thiamine.

Low thiamine levels in the CSF were associated with significantly low CSF 5HIAA (Table 5). Although patients with low thiamine levels had a trend towards having lower folate levels than those seen in patients with normal thiamine, this does not explain the lowering of CSF 5HIAA. Low folate levels cause a lowering of CSF 5HIAA but borderline folate levels do not (Table 4). The mean folate level in the thiamine deficient patients was in the borderline folate range. Differences between subjects with borderline thiamine levels and those with normal levels in general showed the same trend as between subjects with low and normal levels, but were smaller and were not statistically significant.

Because low levels of folate and thiamine in the CSF are associated with increased duration of epilepsy, and thus increased duration of treatment, we looked at whether

TABLE 5 FACTORS ASSOCIATED WITH VARYING CSF THIAMINE LEVELS*

	Variable			*P values*	
	Group 1 *Low thiamine*	*Group 2* *Borderline*	*Group 3* *Normal thiamine*	*1 vs 3*	*2 vs 3*
Age (yrs)	45 ± 14(36)	46 ± 14(43)	44 ± 17(72)	n.s.	n.s.
Lifetime PHT (g)	1540 ± 1370(36)	1530 ± 1400(43)	1040 ± 1150(72)	<0.1	<0.05
Serum PHT (μg/ml)	11.7 ± 6.3(35)	10.6 ± 7.1(42)	11.0 ± 10.5(72)	n.s.	n.s.
CSF PHT (μg/ml)	2.03 ± 1.99(35)	1.59 ± 1.17(41)	1.57 ± 1.77(72)	n.s.	n.s.
Duration of epilepsy (mos)	222 ± 168(36)	203 ± 153(43)	155 ± 166(72)	<0.05	n.s.
No. of grand mal seizures	26.3 ± 38.1(36)	30.6 ± 50.6(43)	16.3 ± 30.2(71)	n.s.	<0.1
No. of complex partial seizures	447 ± 1455(36)	137 ± 411(43)	218 ± 413(72)	n.s.	n.s.
Serum folate (ng/ml)	6.59 ± 4.28(36)	6.43 ± 3.84(38)	6.15 ± 3.91(59)	n.s.	n.s.
CSF folate (ng/ml)	21.9 ± 12.4(36)	29.6 ± 19.7(38)	27.0 ± 11.6(60)	<0.1	n.s.
Blood thiamine (ng/ml)	43.0 ± 12.4(36)	44.6 ± 9.5(43)	48.2 ± 10.9(71)	<0.05	<0.1
CSF thiamine (ng/ml)	15.1 ± 4.3(36)	21.6 ± 1.7(43)	30.9 ± 5.6(72)		
CSF tryptophan (ng/ml)	346 ± 80(36)	312 ± 103(42)	341 ± 119(71)	n.s.	n.s.
CSF tyrosine (ng/ml)	1700 ± 890(36)	2590 ± 2270(41)	1990 ± 1520(69)	n.s.	n.s.
CSF 5HIAA (ng/ml)	16.5 ± 7.4(36)	21.2 ± 10.2(43)	24.4 ± 17.8(71)	<0.005	n.s.
CSF HVA (ng/ml)	38.0 ± 21.6(35)	41.3 ± 22.0(42)	43.4 ± 25.9(69)	n.s.	n.s.
CSF MHPG (ng/ml)	7.68 ± 4.41(36)	8.60 ± 3.99(43)	10.48 ± 7.44(72)	<0.05	<0.1

* All values are given as mean ± SD (no. of patients)

increased length of treatment with PHT might be associated with low CSF 5HIAA. As can be seen from Table 6, longer treatment with PHT is associated with lower folate and thiamine levels, but not with any significant difference in amine metabolite levels. Thus the lowering of CSF 5HIAA is related to low vitamin levels and not length of PHT treatment. Surprisingly, treatment for more than 5 yrs results in a significant elevation in CSF tyrosine.

Variables associated with atrophy (Table 7) give similar results to those for length of treatment. Atrophy is associated with a greater number of seizures, but because of the variability in the number of seizures between patients this difference is not statistically significant. Atrophy is significantly related to greater lifetime dosage of PHT, and greater PHT levels at the time the patients were studied. Patients with atrophy had lower levels of folate and thiamine. Mean CSF thiamine values for patients with atrophy were in the borderline range, whereas the mean CSF folate level was just above the borderline range. Atrophy was associated with lower levels of 5HIAA, HVA and MHPG in the CSF, but these differences were not statistically significant.

DISCUSSION

In this study we looked at the relationship between a variety of interacting variables and CFS amine metabolites. Our results indicate that in treated epileptic patients the main determinants of abnormal amine metabolite levels are PHT intoxication and low levels of folate and thiamine. These three factors seem to act independently. Other factors

TABLE 6 FACTORS ASSOCIATED WITH LENGTH OF PHT TREATMENT*

	Variable			*P values*	
	Group 1 *No treatment*	*Group 2* *Treated less than 5 yrs*	*Group 3* *More than 5 yrs*	*1 vs 2*	*1 vs 3*
Serum folate (ng/ml)	9.00 ± 3.71(28)	5.88 ± 4.04(17)	5.59 ± 3.70(94)	<0.01	<0.001
CSF folate (ng/ml)	36.4 ± 15.2(29)	27.2 ± 17.0(17)	22.9 ± 12.4(93)	<0.05	<0.001
Blood thiamine (ng/ml)	46.6 ± 9.7(35)	46.8 ± 14.4(17)	45.4 ± 11.0(101)	n.s.	n.s.
CSF thiamine (ng/ml)	27.1 ± 5.9 (35)	23.9 ± 9.4(17)	23.7 ± 8.2(99)	n.s.	<0.025
CSF tryptophan (ng/ml)	317 ± 119(34)	294 ± 120(20)	349 ± 95(100)	n.s.	n.s.
CSF tyrosine (ng/ml)	1460 ± 550(34)	1930 ± 1550(19)	2320 ± 1860(98)	n.s.	<0.001
CSF 5HIAA (ng/ml)	20.4 ± 14.6(35)	26.1 ± 25.5(20)	21.0 ± 10.3(100)	n.s.	n.s.
CSF HVA (ng/ml)	35.2 ± 18.7(34)	45.6 ± 28.7(20)	42.5 ± 23.9(97)	n.s.	<0.1
CSF MHPG (ng/ml)	9.50 ± 6.60(35)	10.86 ± 9.69(20)	9.16 ± 5.35(101)	n.s.	n.s.

* All values are given as mean ± SD (no. of patients).

TABLE 7 FACTORS ASSOCIATED WITH CNS ATROPHY*

	Variable			*P values*	
	Group 1 *No atrophy*	*Group 2* *Cerebellar atrophy*	*Group 3* *Cerebellar and cerebral atrophy*	*1 vs 2*	*1 vs 3*
Age (yrs)	41 ± 14(51)	40 ± 13(41)	55 ± 14(44)	n.s.	<0.001
Lifetime PHT (g)	785 ± 1187(51)	1150 ± 1430(41)	1684 ± 1188(44)	<0.01	<0.001
Serum PHT (µg/ml)	7.08 ± 8.33(50)	11.4 ± 8.0(41)	14.2 ± 8.4(43)	<0.025	<0.001
CSF PHT (µg/ml)	0.94 ± 1.62(50)	1.80 ± 1.69(40)	2.14 ± 1.25(43)	<0.01	<0.001
Duration of epilepsy (mos)	127 ± 151(51)	201 ± 149(41)	228 ± 179(44)	<0.05	<0.005
No. of grand mal seizures	15 ± 35(50)	23 ± 35(41)	29 ± 48(44)	n.s.	<0.1
No. of complex partial seizures	134 ± 352(51)	283 ± 584(41)	377 ± 1294(44)	n.s.	n.s.
Serum folate (ng/ml)	7.74 ± 3.98(41)	6.15 ± 3.95(39)	5.44 ± 3.48(40)	<0.1	<0.01
CSF folate (ng/ml)	31.9 ± 15.7(42)	23.9 ± 12.7(38)	25.3 ± 15.7(40)	<0.025	<0.05
Blood thiamine (ng/ml)	46.9 ± 9.2(51)	45.6 ± 11.8(40)	44.4 ± 10.8(43)	n.s.	n.s.
CSF thiamine (ng/ml)	28.3 ± 7.3(51)	22.0 ± 7.9(39)	22.3 ± 7.2(44)	<0.001	<0.001
CSF 5HIAA (ng/ml)	24.2 ± 19.6(51)	20.3 ± 10.6(40)	19.8 ± 10.8(43)	n.s.	n.s.
CSF HVA (ng/ml)	45.4 ± 26.3(48)	39.3 ± 23.8(39)	37.2 ± 19.5(43)	n.s.	n.s.
CSF MHPG (ng/ml)	10.2 ± 7.4(51)	8.41 ± 4.81(40)	8.57 ± 5.36(44)	n.s.	n.s.

* All values are given as mean ± SD (no. of patients). The presence of cerebellar or cerebellar and cerebral atrophy was determined using clinical judgement after inspection of the CT scan, as described in the text.

we studied were length of PHT treatment and atrophy. Different lengths of treatment were not associated with significant differences in amine metabolite levels, although longer treatment results in a trend to higher HVA levels (Table 6). This may have been due to elevated precursor levels as mean CSF tyrosine was 59% higher in patients treated for more than 5 yrs than in patients treated less than 3 yrs. This marked elevation was probably not due to an alteration in the system which transports tyrosine into brain, as tyrosine shares this system with other large neutral amino acids such as tryptophan (Oldendorf and Szabo, 1976), and tryptophan levels did not increase. The most likely explanation is that blood tyrosine levels were elevated. This would suggest lowered levels of liver tyrosine aminotransferase, the principal enzyme catabolizing tyrosine. The effect on tyrosine is not indicative of a general effect on amino acids as PHT causes a small (15%) lowering of plasma tryptophan (Pratt *et al.*, 1984).

Increasing length of treatment was associated with low levels of both folate and thiamine, as might be expected. However, in patients treated for more than 5 yrs, the CSF levels of these vitamins were only in borderline range. This explains why increasing length of treatment had no significant effect on amine metabolite levels, as borderline levels do not lower amine metabolite levels.

Data for patients with cerebellar atrophy or cerebellar and cerebral atrophy were similar to those for length of treatment, except that patients with cerebral atrophy were older than those with no atrophy (Table 7). Atrophy was not associated with significant alterations in the levels of amine metabolites in the CSF. Atrophy was associated with lower levels of folate and thiamine but, as with length of PHT treatment, mean levels of folate and thiamine in the CSF of patients with atrophy were only in the borderline range.

CSF levels of the vitamins revealed that 35% had low folate, and 24% had low thiamine levels. None of the patients exhibited frank nutritional deficiencies, but it is likely that suboptimal dietary intake played some role in these results. However, in patients with low levels of one of the vitamins, mean CSF and plasma levels of the other did not reveal low levels, although borderline levels were seen. Thus although there may have been some overlap between low folate and thiamine levels, in general they were separate phenomena. Although we found a relationship between thiamine and folate levels in both blood and CSF of alcoholic patients (Botez *et al.*, 1982*c*), deficiencies of folate and thiamine were separate phenomena in our preliminary study on epileptics (Botez *et al.*, 1982*a*).

The folate deficiency associated with anticonvulsant treatment is a well studied phenomenon (Reynolds, 1973, 1979) and seems to be due to decreased absorption of folate from the diet (Rosenberg *et al.*, 1979; Smith and Obbens, 1979). This is consistent with our finding that the mean decline in folate levels in the group with low folate levels, relative to the normal folate group (Table 4), was similar for serum (54% decline) and CSF (66% decline). The incidence of low folate in the present study (35%) is in good agreement with the results on folate deficiency in previous studies (Wells and Casey, 1967; Reynolds *et al.*, 1969; Streiff *et al.*, 1972; Reynolds *et al.*, 1975; Krause *et al.*, 1982).

The relatively high incidence of low thiamine levels associated with PHT treatment confirms the results of our preliminary study (Botez *et al.*, 1982*a*). The mechanism for this effect is unknown. The decline in CSF thiamine (51%) in the low thiamine group was much greater than the decline in blood thiamine (11%). There are three possible explanations for this. First, the deficient thiamine levels may have been due to marginal dietary intake and/or PHT induced malabsorption. As the samples of blood and CSF were taken several days after the patients were admitted to hospital, the hospital diet may have helped to replete low blood thiamine levels, but the time interval may have been too short to raise CSF thiamine. If this explanation is true it would imply that the hospital diet repleted blood thiamine much faster than serum folate. Secondly, PHT may have inhibited uptake of thiamine into the CNS and CSF. Thirdly, low CSF thiamine may have occurred as a result of a decline in the CNS, and more specifically, the cerebellar thiamine content, which in turn was due to atrophy. The cerebellum has the highest thiamine turnover of all anatomical structures in the CNS (Rindi, 1982; Reggiani *et al.*, 1984; Rindi *et al.*, 1984). Patients with cerebellar atrophy might well show low CSF thiamine levels resulting from the cerebellar damage itself due, at least in part, to the affinity of PHT for Purkinje cells (Bernocchi *et al.*, 1983). In favour of this interpretation is the fact that there is a highly significant ($P < 0.001$) decline in CSF thiamine in patients with cerebellar atrophy, compared with those with no atrophy (Table 7), even though blood thiamine is not significantly different in the two groups.

Anticonvulsants can increase rat brain 5HT (Bonnycastle *et al.*, 1957; Anderson *et al.*, 1962; Jenner *et al.*, 1975). Chadwick *et al.* (1977) studied the effect of anticonvulsant treatment on CSF tryptophan and 5HIAA in epileptic patients. Whereas normal levels were found in some patients, elevated amounts of both compounds were found in subgroups of the patients. Elevated levels of tryptophan and 5HIAA were associated with treatment with several anticonvulsant drugs, while drug intoxication increased only CSF 5HIAA. In our study the maximum number of anticonvulsant drugs used was two, so we were unable to check the idea that treatment with several anticonvulsants leads to increased CSF indoles. However, our data agree entirely with the conclusion of Chadwick *et al.* (1977) that anticonvulsant intoxication is associated with elevated CSF levels of 5HIAA, but not tryptophan (Table 3).

Previously we have found low levels of 5HIAA in the CSF of patients who were folate deficient due to inadequate diet or malabsorption, and who exhibited folate-responsive mental symptoms (Botez *et al.*, 1982*b*). Bottiglieri *et al.* (1986) found a significant positive correlation between CSF 5HIAA and red cell folate in a group of depressed patients one-third of whom were folate deficient. The present study provided further evidence that low folate levels can result in lowered CNS 5HT turnover (Table 4). We also found low CSF HVA associated with folate deficiency. Bottiglieri *et al.* (1986) failed to find an association between CSF HVA and red cell folate. We originally studied the relationship between 5HT and folate deficiency because of the finding that the regional distribution of 5HT in rat brain is similar to that of 5-methyltetrahydrofolate (Korevaar *et al.*, 1973). We found that folate deficient rats had low brain 5HT, but normal brain dopamine (Botez *et al.*, 1979). In the present study the low HVA may have occurred as a secondary result of low 5HIAA, as the levels of the 2 metabolites in human CSF usually exhibit a high degree of correlation (Ågren *et al.*, 1986).

Thiamine deficiency is known to cause impairment in the cerebellar 5HT system (Plaitakis *et al.*, 1978). In a preliminary study of thiamine deficient patients we found low CSF 5HIAA in 3 out of 5. In all 3, thiamine treatment caused a marked increase in CSF 5HIAA (Botez *et al.*, 1982*b*). The present study confirms a significant lowering of CSF 5HIAA in patients with low thiamine levels (Table 5). We found no lowering of CSF HVA associated with low thiamine, but we did find a significiant decline in MHPG. This agrees with the significant lowering of noradrenaline in the olfactory bulb and cortex of thiamine deficient rats (Mair *et al.*, 1985), and the low CSF MHPG in patients with Korsakoff's psychosis (McEntee and Mair, 1978).

The results of our present study help to reconcile some of the divergent conclusions from previous studies on the effect of anticonvulsants on biogenic amine metabolism. Thus the increase in CSF 5HIAA reported by Chadwick *et al.* (1977) seems to be related to anticonvulsant intoxication. The low levels of 5HIAA or HVA in treated epileptic patients reported by Shaywitz *et al.* (1975), Young *et al.* (1980) and Kalfakis and Markianos (1987) were presumably due to low levels of folate and/or thiamine, although vitamin levels were not measured in these studies.

One important issue for further study is whether the lowered levels of biogenic amine metabolism caused by the low vitamin levels lead to lowered biogenic amine function. Several studies do suggest that altered neurotransmitter function might occur. Thus Onodera *et al.* (1981) obtained evidence that muricide induced by thiamine deficiency in rats is mediated by lowered 5HT function. Mair *et al.* (1985) also looked at behaviour in thiamine deficient rats and concluded that deficits in learning and memory were due to lowered noradrenaline levels. This is consistent with the finding that memory impairments in patients with Korsakoff's syndrome is correlated significantly with reductions in CSF MHPG (McEntee and Mair, 1978). We have found that the lowering of CSF 5HIAA which can result from dietary folate deficiency is associated with mental symptoms including neuropsychological changes and a lowering of mood (Botez *et al.*, 1982*b*). In psychiatric patients with various diagnoses, low CSF 5HIAA is a marker of suicide attempts and successful suicide (Åsberg *et al.*, 1987). Follow-up studies concerned with the mortality of epilepsy indicate that the suicide rate is increased (Barraclough, 1987), suggesting that anticonvulsant-induced lowering of folate and thiamine levels may lead to decreased brain 5HT and increased vulnerability to psychosocial stressors. Thus low vitamin levels in epileptic patients may lead to neuropsychological deficits and alterations in affect.

ACKNOWLEDGEMENTS

This work was supported by grants from the Medical Research Council of Canada (to S.N Y.), and from Fonds pour la Formation de Chercheurs et l'Aide a la Recherche (grant no. EQ3417) and the Bernard Dubois fund (to M.I.B.). The authors are grateful to Drs Louise Charron, Luc Marchand, Adolpho Perez de Leon and Andrée Ross-Chouinard for referring patients under their care. Dr Ezzedine Attig, a resident in neurology, helped the senior author in the follow-up and radiological assessment of the patients. Mr Marc Dumont carried out the statistical analyses. Antonietta Gasparini and Ana de Oliveira provided excellent technical assistance.

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(Received July 27, 1988. Revised March 16, 1990. Accepted March 30, 1990)

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Printed in Great Britain

UCH

ISSN 0006-8950

Acc 12/7/93

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Brain (ISSN 0006-8950) is published bi-monthly by Oxford University Press, Southfield Road, Eynsham, Oxford OX8 1JJ, England. Annual subscription price in USA $195.00 including air speed delivery. Second class postage paid at Jamaica, NY 11431.

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SELECTIVE IMPAIRMENT OF MEMORY AND VISUAL PERCEPTION IN SPLENIAL TUMOURS

by PETER RUDGE *and* ELIZABETH K. WARRINGTON

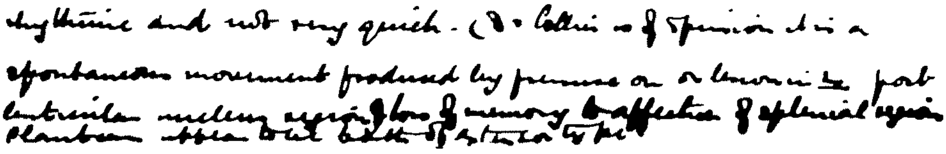
(*From the National Hospital, Queen Square, London, UK*)

SUMMARY

The neuropsychological abnormalities found in 9 patients with tumours involving the splenium of the corpus callosum are described. The outstanding features of their cognitive deficits were a severe memory deficit and visual perception impairment in the presence of relatively intact intellect. It is argued that (1) the amnesia is due to damage to the fornix where that structure is closely applied to the splenium and that it is the result of a disconnection between the frontal and temporal lobes, although the possibility that damage to more than one structure, for example, retrosplenial cortex and fornix, cannot be excluded; (2) there is a dual pathway for visual object recognition, one of which passes directly to the dominant hemisphere for semantic analysis and the other via the nondominant hemisphere for prior perceptual analysis. Further, it is postulated that there is a subcortical as well as a callosal route between the hemispheres that is important for visual object recognition.

INTRODUCTION

Corpus callosum tumours produce marked changes in mentation early in their evolution, but there is little recognition in the modern literature of the highly specific defects associated with lesions in different parts of that structure. In fact, tumours of the splenium produce selective impairment of memory. This has recently been emphasized by Valenstein *et al.* (1987) but was part of conventional wisdom in the early part of this century. For example, Ironside and Guttmacher (1929) reviewed 14 patients with corpus callosum tumours and showed that splenial lesions invariably caused an early and severe memory loss while more anterior lesions resulted in apathy and apraxia. A particularly instructive case from their publication is Case 5 of whom Collier, under whom the patient was admitted to the National Hospital, considered (fig. 1) that the splenium must be involved to account for the memory impairment, an observation that proved well founded at autopsy, clearly indicating that this was an accepted clinical fact at that time. There



rhythmic and not very quick. (Dr Collier is of opinion it is a
spontaneous movement produced by pressure on a tumour in the post
lenticular nucleus region) loss of memory due to affection of splenial region
[illegible]

FIG 1 Photograph from original National Hospital notes made by Dr Wyllie, resident to Dr Collier, on rounds on March 6, 1922 concerning Case 5 of Ironside and Guttmacher (1929) Collier states that the 'loss of memory (due) to affection of splenial region'

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are numerous other papers from this period emphasizing the same point (*see* e.g., Cramer, 1936; Trescher and Ford, 1937) but few have appeared since (Schlesinger, 1951). While reviewing, from a neuropsychological point of view, a number of patients with corpus callosum tumours it became apparent that a visual perceptual deficit was also a constant feature of the clinical picture, provided the lesion was posterior. This paper presents clinical and neuropsychological data on 9 patients with splenial tumours who have been studied over the past 13 yrs.

METHODS

Selection of patients

Patients were selected by reviewing all those seen in the Neuropsychology Department of the National Hospital in the preceding 13 yrs in whom a final diagnosis of bilateral tumours was made. Of these, 10 had a tumour of the posterior corpus callosum, 1 patient had no vision and will not be discussed further, this report concerns the remaining 9. CT scans of the brain (EMI 5005, 1010, GE 8800, 9800 depending on date) were located for all patients but in 1 only a limited number of sections was available (Case 5). Magnetic resonance imaging (MRI) using a 0.5 Tesla Picker super-conducting system was obtained in 2 subjects.

Neuropsychological testing

In most cases neuropsychological testing was carried out before a clinical diagnosis was made, i.e., before any radiological tests were done. The patients were assessed by a small team of psychologists who administered tests of intelligence, memory, language, literacy skills and perception, clinical considerations dictating the precise choice of tests in each case; consequently, tests to detect disconnection were not routinely performed.

Verbal and performance intelligence quotients (IQ) were prorated from the scores of 4 verbal (arithmetic, similarities, digit span and vocabulary) and 3 performance tests (picture completion, picture arrangement, block design) of the Wechsler Adult Intelligence Scale (WAIS) and an estimate of premorbid intelligence was obtained (except in the dyslexic patients) from their reading skills (National Adult Reading Test, NART).

Memory was assessed with the Recognition Memory Test which has a verbal and visual version. The verbal version comprises 50 stimulus words and recognition memory is estimated by presenting each with a paired distractor word and demanding a forced choice from the patient. The comparable visual version consists of 50 stimulus faces (Warrington, 1984).

Language and literacy skills were assessed with a Graded Naming Test (GNT) comprising 30 black and white drawings with a range of frequency that enabled an IQ equivalent to be calculated by prorating from an equivalent vocabulary scaled score (7 patients) (McKenna and Warrington, 1983); in 2 patients the simpler Oldfield and Wingfield (1965) test was administered.

Visual perceptual skills were estimated by asking the patient to identify a series of 20 objects photographed from an unusual angle, for example, a bucket viewed from directly above (all patients) or to identify a series of letters in which parts of the image had been randomly deleted, the fragmented letter test (5 patients). For further details of these tests, *see* Warrington and James (1988).

RESULTS

Patients

There were 5 men (aged 46–71, mean 57 yrs) and 4 women (aged 57–73, mean 62 yrs) (Table 1). One patient was left-handed but speech was proven to be dependent on the left hemisphere by the amytal test. All were considered to have a tumour of the corpus callosum on radiological examination and all but 1 have died. Histological proof

TABLE 1 DEMOGRAPHIC DATA AND INTELLECTUAL PERFORMANCE OF PATIENTS

Case	*Age (yrs)*	*Sex*	*Handedness*	*Verbal IQ*	*Performance IQ*	*NART*	*GNT equivalent IQ*
1	73	F	R	115	102	114	115
2	71	M	R	87	78	Dyslexic	95
3	59	F	R	99	83	111	95
4	52	M	R	117	93	117	120
5	56	M	R	99	108	100	100
6	46	M	L*	123	110	120	115
7	57	F	R	99	87	Dyslexic	**
8	62	M	R	101	70	115	**
9	65	F	R	95	58	Dyslexic	90

* Amytal test, left-handed but speech in left hemisphere ** *See* text

of the diagnosis was obtained in 5 patients, including the case still alive; 4 had a malignant astrocytoma and 1 a lymphoma.

Symptoms

A strikingly consistent clinical picture was present in these patients. All had an obvious memory impairment at the time of initial examination and in 8 it was the presenting symptom. In all cases the memory impairment was of such severity that the patient was unable to give a history; this provides strong clinical evidence of a profound retrograde amnesia. Without exception the relatives noted it and often described the patient as confused or disorientated. This symptom was always less than 3 months duration. Seizures occurred in 2 patients as a presenting symptom and in 1 of these the seizure coincided with the onset of the memory impairment. Only 1 patient presented with headache.

Neurological signs

On neurological examination only 1 patient had papilloedema; 4 had a definite homonymous hemianopia (2 to the left) and 2, in whom formal fields were not plotted, had an equivocal right hemianopia; 3 patients had normal visual fields; 5 patients had normal motor and sensory examination in the limbs, 4 of whom had a hemianopia; 4 patients had a minimal or mild hemiparesis and 3 of these had minimal sensory signs on the same side. None was dyspraxic but 2 had evidence of unilateral neglect.

Cognitive function

Intellectual performance. Eight of the 9 patients obtained at least an average score on the verbal scale of the WAIS (Table 1). Six were tested on the NART and all obtained reading IQ equivalents of 100 or more. In only 2 (Cases 3, 8) was there a significant discrepancy between their reading IQ equivalent and verbal IQ, indicating a mild deterioration from their previous level. Performance IQ was variable, 3 patients scoring well below average.

Memory. The age-corrected percentile scores obtained are given in Table 2. Two patients (Cases 2, 9) were so grossly amnesic that a particularly easy, multiple choice quantitative test of recognition memory for distinctive coloured photographs (Warrington, unpublished) were administered and both patients scored well below the first percentile.

TABLE 2. MEMORY AND VISUAL PERCEPTION

	Recognition memory		*Perception*	
Case	*Words*	*Faces*	*Unusual views*	*Fragmented letters*
1	<5%	=5%	<5%	<5%
2	NT	*	<5%	NT
3	<5%	25–50%	<5%	NT
4	<5%	=5%	<5%	=5%
5	<5%	=25%	=5%	=5%
6	>50%	=5%	=25%	<5%
7	<5%	25–50%	<5%	NT
8	NT	<5%	<5%	NT
9	<5%	*	<5%	<5%

* *See* text. NT = not tested.

The amnesia of Cases 2 and 8 was of sufficient severity to preclude quantitative assessment of verbal recognition memory (for more details of Case 2, *see* Costello and Warrington, 1987). In the remaining 6 patients, visual and verbal recognition memory was profoundly impaired in 2, visual recognition memory more severely affected in 1 and verbal recognition memory more affected in 3.

Language and literacy skills. Seven patients tested on the Graded Naming Test obtained satisfactory scores while the 2 assessed on the simpler Oldfield and Wingfield object naming test made few errors, indicating relatively preserved nominal skills. These findings effectively eliminate a visual associative agnosia. Three patients were not assessed on the NART because of dyslexic difficulties; 2 of these had a mild left neglect dyslexia.

Perceptual and spatial skills. Eight of the 9 patients scored at or below the fifth percentile on the unusual views test (Table 2). Five attempted the fragmented letters test and all, including the patient who performed satisfactorily on the unusual views test, scored at or below the fifth percentile.

CT scan and MRI findings

On CT scanning and MR imaging all tumours were large and all involved the splenium (figs 2–4). Adjacent structures were involved in all patients but there was considerable variation between subjects. The corpus callosum anterior to the splenium was involved in all but 1 patient, the tumour extending up to the middle of the body in the patient with the most extensive mass. The tumour always extended into the hemispheres, affecting the occipital lobes in all cases, the parietal lobes in 8 and the temporal lobes in 4, but this extension was markedly asymmetric in all but 1 patient (Table 3). The extension into the left hemisphere was the greater in all but 3 cases. The hippocampus and thalamus were spared in all subjects.

DISCUSSION

The patients described here demonstrate that tumours of the splenium and posterior part of the corpus callosum present a distinct, perhaps diagnostic, clinical picture in which there is a selective severe and early memory impairment, few formal neurological

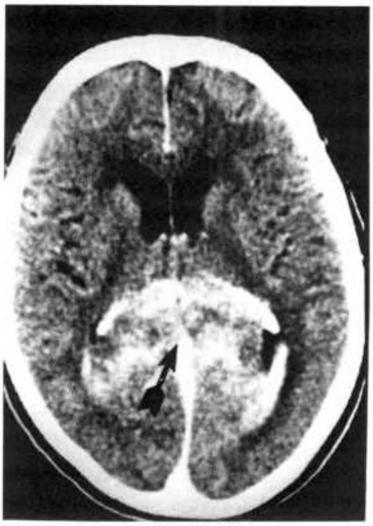

FIG. 2. Enhanced CT scan at about the level of splenium in *Case* 9. Note extensive mass extending into both hemispheres. Left of patient on right of illustration.

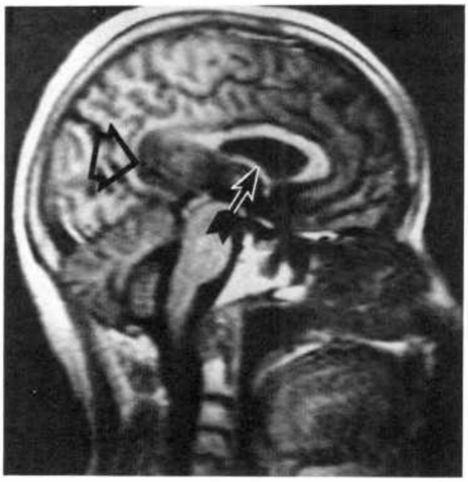

FIG. 3. T_1-weighted MRI in sagittal plane illustrating splenial tumour (open arrow) and fornix (closed arrow). Note the fornix disappears into the tumour.

signs other than a field defect, and a marked visual perceptual deficit in the absence of overt signs of raised intracranial pressure. Many current neurological texts give scant details of the clinical picture in corpus callosum tumours other than saying the patients have a high incidence of mental changes without being more specific (*see*, for example, Walton, 1977). This is surprising in view of the large amount of work on corpus callosum section, much of which has concentrated upon the capabilities of individual hemispheres in patients with long-standing and intractable epilepsy. The paucity of data is, no doubt, partly due to the ready availability of noninvasive imaging techniques developed in the past two decades, rendering full clinical evaluation of the patient less important in

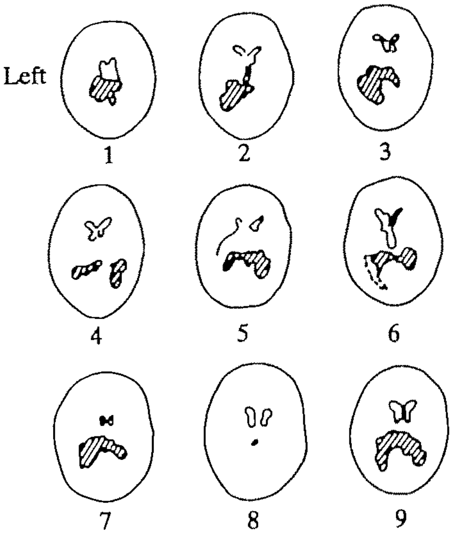

FIG. 4. Tracings of outlines of all tumours at level of splenium. Some of the scans are horizontal, others up to 25° from the horizontal. All are illustrated as if viewed from the vertex The section closest to the splenium has been selected. Asymmetry at this level does not necessarily correspond to overall asymmetry (*see* Table 3)

TABLE 3 SCAN FINDINGS

Case	*Symmetry*	*Parietal*	*Temporal*	*Corpus callosum**
1	L>R	L>R	N	Posterior half
2	L≫R	L	L	Splenium alone
3	L>R	L	L	Posterior third
4	L>R	L	R	Posterior third
5**	L>R	L>R	–	Posterior half
6	R>L	R	R	Posterior third
7	L>R	L≫R	N	Posterior third
8	R>L	R>L	N	Posterior third
9	L=R	N	N	Posterior quarter

* All patients had involvement of splenium. ** Incomplete set of scans available L = left, R = right involvement, N = normal

determining an anatomical diagnosis. However, failure to examine the patient in detail discards much information of potential practical and theoretical interest.

The memory impairment was particularly striking in the patients described here. The abnormality could involve either verbal or visual memory or both. Why does this occur? It does not appear to be a trivial consequence of raised intracranial pressure in that only 1 patient had papilloedema, there was no obtundation and cognitive function was relatively preserved apart from the amnesia and visual perceptual deficits. Similarly, any general effect on cerebral metabolism due to the tumour, so commonly seen when the cerebral blood flow and glucose degradation are studied with PET scanning, is unlikely to be the cause of such specific defects unless memory and visual gnosis are exquisitely sensitive to such impairments; and it seems highly implausible that it is only when these metabolic abnormalities are induced by tumours of the posterior part of the corpus callosum and nowhere else that this picture emerges. In the following paragraphs we will argue that it is damage to the fornix subjacent to the splenium that causes the amnesia in splenial tumours. This argument depends crucially upon memory being independent of the corpus callosum itself. Data are limited but there is no good evidence that surgical section of the corpus callosum causes amnesia in most cases (Clark and Geffen, 1989) and at the clinical level it is rare for patients or their relatives to notice any memory impairment after such operations. If this is accepted, what structures are involved?

There is a large body of evidence that medial parts of the temporal lobes are important for memory and that removal of the hippocampus and adjacent structures bilaterally causes profound amnesia (Scoville and Milner, 1957). A major pathway between the hippocampus and other areas of the brain is the fornix. This runs from the hippocampus to the mamillary bodies from which pathways pass to the anterior nuclei of the thalamus—the mamillothalamic tract. The anterior thalamic nuclei connect with the frontal cortex. Damage to the medial part of the thalamus and mamillary bodies sometimes causes amnesia but precisely which structures are involved is unclear. On balance it appears that the mamillothalamic tract is crucial, at least in vascular disease causing amnesia (von Cramon *et al.*, 1985). Victor *et al.* (1989) in their studies of patients with alcoholic Korsakoff psychosis thought that the medial thalamic nuclei are important but unfortunately, despite extensive histological examination, they could not exclude damage to the fibre pathways including the mamillothalamic tract. Further, the effects of toxins such as alcohol are widespread and are not ideally suited precisely to define anatomical bases of a disturbance. There is no consensus on the effects of fornix section (Woolsey and Nelson, 1975) but cases exist where amnesia did result from damage to the anterior part of the fornix (Zeman and King, 1958; Brion *et al.*, 1969). One of the problems in interpreting these data is that most cases had lesions of the third ventricle, especially colloid cysts. Since such lesions can affect medial thalamic structures it is conceivable that it is this damage, rather than fornix section, that causes the amnesia. Alternatively, there is evidence that not all the fibres from the hippocampus course to the mamillary bodies in the fornix; some do not pass through the anterior pillars but diverge direct to the thalamus (Valenstein and Nauta, 1959). This also could account for the variable effects on memory observed after section of the anterior pillars of the fornix, since a complete disruption of the hippocampal-derived fibres may not be achieved. In contrast more posterior damage to the fornix will disrupt all the fibres in the fornix originating in the hippocampus and destined for the mamillary bodies and thalamus. Such a lesion, if complete, should reliably cause amnesia.

There is now good evidence that there are multiple memory systems and many subdivisions have been suggested. Neurologists have tended to use the temporal domain, referring to short-term (less than 15 s, small capacity) and long-term (long duration, large capacity) memory and there is merit in this at the anatomical level in that the two seem to be dependent on different structures. However, Tulving (1972) pointed out that long-term memory was a complex phenomenon and lumping together all memories other than those defined as short-term is a gross simplification. He drew attention to the fact that long-term memory comprised what he termed *semantic memory* in which a corpus of facts is shared by groups of individuals, such as memory for concepts, words, and objects, and *episodic* or *event memory* of autobiographical data. Warrington and Weiskrantz (1982) developed Tulving's basic distinction between episodic/event and semantic memory systems and postulated that in normal subjects there is a *cognitive mediational memory system* which provides the individual with a long-term record of events. Furthermore, they proposed that amnesia is a disconnection syndrome. Briefly, they argued as follows. Structures in the temporal lobes subserve semantic memory while structures in the frontal lobe subserve the cognitive mediational memory system or some component of it. If these two systems are disconnected the cognitive mediational memory system would have no meaningful input to drive either the encoding of new events or retrieval of old ones. Thus memory for ongoing events would be without content and memory for remote events inaccessible. The patient would be amnesic. Damage anywhere along the pathways connecting the temporal and frontal lobes should impair the operation of the cognitive mediational memory system.

If such a disconnection hypothesis is accepted why do patients with posterior, but not anterior, corpus callosum lesions have amnesia? The fornix is remote from the corpus callosum as its anterior pillars ascend but then become closely applied, indeed inseparable from, the posterior part of the corpus callosum, especially the splenium, before descending into the temporal lobe. Any tumour of the splenium will inevitably damage the fornix (*see* fig. 3) disrupting the thalamohippocampal connections via this route and should thus cause amnesia on the disconnection hypothesis; a more anterior corpus callosum lesion will spare the fornix and consequently memory. Such a view is the most economical concerning the cause of the amnesia and it is supported by the cases described as well as the example of a tumour arising from the fornix itself beneath the splenium (Heilman and Sypert, 1977). However, tumours of the splenium inevitably cause damage to other structures including the indusium griseum and retrosplenial cortex, both of which have extensive connections with the diencephalon, frontal and temporal cortex (Robertson and Kaitz, 1981; Mufson and Pandya, 1984). Valenstein *et al.* (1987) have argued that these connections are at least as important as the fornix for the amnesia seen in their patient with a splenial lesion (an angioma with haemorrhage). They were forced to this conclusion as they felt that the evidence that fornix lesions alone caused amnesia was not persuasive in man and they were impressed that in monkeys both pathways emanating from the temporal lobe—fornix and amygdalofugal—had to be destroyed to obtain significant amnesia (Bachevalier *et al.*, 1985). It is not possible to resolve this problem at present since virtually all naturally occurring lesions in the region of the splenium will inevitably damage both the fornix and retrosplenial cortex and this particularly applies to tumours. While it may be necessary to have more than one structure damaged to cause amnesia, as in nonhuman primates, it is nevertheless interesting that patients undergoing midline section of the splenium, which will damage the fornix even if the section is strictly midline, often get a temporary, and only occasionally long-lasting amnesia (Akelaitis, 1942; Clark and Geffen, 1989).

One final point concerning memory impairment in patients with splenial tumours was the fact that verbal and visual memory were not invariably affected to an equal extent. There is a considerable body of evidence that left-sided cerebral hemisphere lesions cause impairment of verbal memory while right-sided lesions cause visual memory loss (e.g., Milner, 1974, 1982; Warrington, 1984). The fact that a lesion centred on the midline did not cause equal loss is at first surprising. Although there was asymmetry in all but one of the tumours in our cases, there was no correlation between the type of memory loss and the asymmetry of the lesion. It is clear that involvement of the temporal lobes was not important as it only occurred in 4 patients. Further, in 2 of these with right temporal lobe involvement, 1 had selective visual memory impairment and 1 impairment of both visual and verbal recognition memory. It is possible that the greater involvement of visual or verbal memory was due to incomplete and asymmetric destruction of the fornix. Tucker *et al.* (1988) have recently described a patient with a left fornix lesion in whom there was selective verbal memory loss suggesting that asymmetric damage to the fornix might explain our results. It should be emphasized, however, that in 5 of the 9 patients in this series both verbal and visual memory were equally bad, a result expected from an essentially midline lesion.

The second major cognitive deficit observed in this series of patients was their impairment of visual perception on the unusual views and the fragmented letters tests. The evidence both from group and single case studies indicates that such impairments are associated with right posterior cerebral damage. Moreover, impairments of visual perception are held to implicate a perceptual stage of processing that is prior to, and independent of, semantic analysis. This latter is considered a subsequent stage in which meaning is assigned to an object and which is lateralized to the left hemisphere. Following Lissauer (1890), impairments in these two compartments of object recognition are termed apperceptive and associative agnosia, respectively. A serial type of organization of these components, if not explicitly stated, is implicit in a number of accounts of visual object recognition (e.g., De Renzi *et al.*, 1969; Warrington and Taylor, 1978; Ratcliffe and Newcombe, 1981).

One difficulty with such a model is how to account for the selective impairment of apperception in patients in whom there was no evidence of an associative agnosia. Yet this appears to be the case. Warrington and James (1988) described 3 cases with right hemisphere lesions in whom pronounced deficits of visual perception were documented. At the same time these patients scored at a normal level on exacting tests that probed knowledge of visual object meaning. Their data called into question a simple linear account of the procedures underpinning the full perceptual and semantic specification of an object. Consequently, they postulated that the apperceptive stage might be an optional rather than an obligatory component of object recognition. If this is correct the left hemisphere systems responsible for semantic analysis could receive input, not only from the perceptual processing systems of the right hemisphere, but also directly from the early visual sensory processing systems subserved by the striate and prestriate cortex of both hemispheres. Indeed, they speculated that there were two routes to meaning (fig. 5A). There was, however, no commitment in their analysis, or in that of Warrington and Taylor's (1978) previous serial model, as to whether this optional route that 'connected' perceptual processing systems of the right hemisphere to the semantic processing systems of the left was transcallosal or via a subcortical route. The evidence from the present series of patients suggests that the route could well be callosal.

A characteristic finding observed in 8 of the 9 patients in this series was that, whereas there was no difficulty in identifying photographs of objects presented in their prototypical canonical view, representations from unusual angles were not recognized, nor were fragmented letters, although 6 of the patients could read well and the other 3 could identify single letters without difficulty. The fact that damage to the corpus callosum was the common factor in all patients bears witness to it being this structure that connects the output of the right hemisphere perceptual systems with the left hemisphere semantic processing systems, a hypothesis that is eminently testable prospectively. At the same time the observation that perceptual deficit in these patients was only for ambiguous

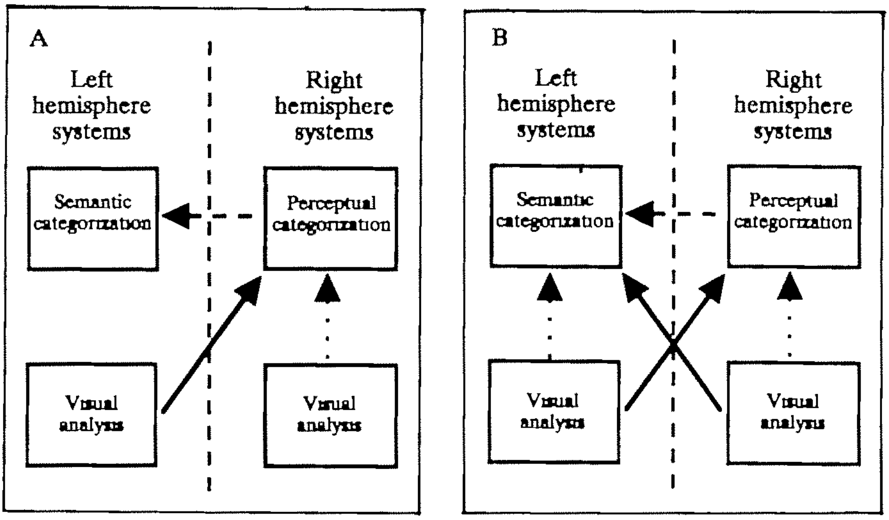

FIG 5 Diagrams representing models of visual perception. A, the model of Warrington and colleagues B, the model proposed in this paper. Note that information can pass from the right to the left hemisphere by both a callosal and subcortical route in model B *See* text for further explanation. Arrow and dashed line = corpus callosum. Arrow and continuous line = subcortical.

or degraded visual stimuli gives credence to the notion of 2 routes to recognition. Furthermore, the evidence from the 2 patients who had a right homonymous hemianopia indicate that this second direct route to the left hemisphere semantic systems may well be subcortical since the right homonymous hemianopia ensures that early visual processing can only be achieved by striate and peristriate structures in the right hemisphere. Although these patients had a perceptual deficit that could be elicited with tests that manipulated perceptual difficulty, their visual gnosis was normal. Thus we would argue that the output of early visual processing was available to the left hemisphere semantic systems and that this direct route must be subcortical (fig. 5B). This deficit has not been described before in the numerous studies of corpus callosum section, presumably because all have been concerned with the relative efficiency of the left or right hemispheres in recognizing unambiguous images.

While the above hypothesis can explain the visual perceptual defect, one should not lose sight of the fact that the tumours in this series were large and involved the parietal lobes in 8 of 9 cases. However, the right parietal lobe, an area known to be important for visual perception, was involved in only 5 cases, and in 1 patient neither parietal lobe was involved, yet performance on unusual views was extremely poor in all. Clearly the possibility exists that remote effects of the tumours played a part but for the same reasons enumerated above concerning the amnesia, it is unlikely that they were important in causing this focal deficit.

In conclusion, patients with posterior corpus callosum tumours present a characteristic clinical picture of rapidly evolving memory loss and a selective impairment of visual perception. It is suggested that this could be explained by a double disconnection, on the one hand between frontal and temporal cortex (amnesia) and on the other, between the hemispheres (apperceptive agnosia).

ACKNOWLEDGEMENTS

The authors are indebted to Merle James for allowing access to her diagnostic index of patients seen in the Neuropsychology Department of the National Hospital, Queen Square, and to all members of that department who tested the patients. We wish to thank Professor Tim Shallice for his helpful criticism and advice during the preparation of this paper.

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(Received September 19, 1989. Revised February 6, 1990. Accepted February 13, 1990)

Brain (1991), **114**, 361–379

THE CONTRIBUTION OF MEDIAN NERVE SEPs IN THE FUNCTIONAL ASSESSMENT OF THE CERVICAL SPINAL CORD IN SYRINGOMYELIA

A STUDY OF 24 PATIENTS

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SUMMARY

Since the early study of Halliday and Wakefield (1963) it has generally been agreed that scalp somatosensory evoked potentials (SEPs) are normal in patients with dissociated loss of pain and temperature sensation. Up to now a few patients with abnormal spinal N13 and preserved scalp P14 and N20 have been reported in the literature, but there is no firm evidence, based on group data, that this dissociation can be related to any form of dissociated sensory loss.

We studied median nerve SEPs in 24 patients with syringomyelia documented by CT scan or MRI. For the recording of the cervical N13 we used a Cv6 anterior cervical montage, which cancels the potentials generated above the foramen magnum and enhances the amplitude of N13. Scalp far-field and early cortical SEPs were recorded using a noncephalic reference electrode. Since N13 pathological changes are mainly changes in amplitude we measured the N13/P9 amplitude ratio in normal subjects and patients and found that it was a reliable index to quantify the amplitude decrease of N13. Absent or reduced N13 was observed in 40 median nerve SEPs (83%) in conjunction with normal P14 and N20 in 30 SEPs. Thus the dissociated loss of the cervical N13 was identified as the most conspicuous SEP feature in syringomyelia. A significant correlation was found between abnormal N13 and loss of pain and temperature sensations, whereas P14 abnormalities correlated well only with loss of joint and touch sensations. In the median nerve territory, sensation was either normal (6 cases) or lost only for pain and temperature (24 cases) when SEPs showed abnormal N13 and normal P14. Although it does not directly reflect the postsynaptic activity of spinal cells receiving their inputs from Aδ and C fibres the N13 potential proved to be a reliable index of spinal cord grey matter dysfunction in syringomyelia.

INTRODUCTION

The notion that recordings of somatosensory evoked potentials (SEPs) to stimulation of the median nerve do not explore the spinothalamic pathways has been documented by numerous experimental and clinical studies (Halliday and Wakefield, 1963; Giblin, 1964; Larson *et al.*, 1966; Namerow, 1969; Nöel and Desmedt, 1975; Cusick *et al.*, 1978; Schieppati and Ducati, 1981). Conversely SEP abnormalities correlate well with loss of joint and cutaneous sensations in circumscribed lesions of the cervical cord (Stöhr *et al.*, 1982), posterior thalamus (Mauguière *et al.*, 1983*a*; Mauguière and Desmedt, 1988) and parietal cortex (Mauguière *et al.*, 1983*b*). Therefore median nerve SEPs are not usually viewed as a useful investigation in patients with syringomyelia and loss of

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temperature and pain sensations in upper limbs. There are, however, two sets of strong arguments suggesting that the posterior neck N13 negativity elicited by electrical stimulation of the median nerve has a fixed transverse generator in the lower cervical cord and reflects the response of dorsal horn neurons to nonnoxious inputs. First, it has been demonstrated, using surface as well as direct spinal recordings during surgery, that N13 reaches its maximal amplitude close to the entry zone of the 5th–7th cervical roots and has a fixed latency from Cv7–Cv2 spinous processes in multichannel recordings; moreover, N13 reverses its polarity when recorded at the anterior aspect of the neck or of the cord itself (Desmedt and Cheron, 1981*a*; Mauguière, 1983; Emerson *et al.*, 1984; Iragui, 1984; Jeanmonod *et al.*, 1989). Secondly, N13 remains unaffected in patients with cervical cord lesions above Cv4 level, whereas later SEP components P14 and N20, generated above the foramen magnum, are abnormal or absent (Mauguière and Courjon, 1981; Mauguière *et al.*, 1983*c*; Mauguière and Ibañez, 1985).

The characteristics of N13 in humans are thus very similar to those of the N1 spinal potential described in animal studies, which reflects the postsynaptic neuronal response to inputs conveyed by group I and II peripheral afferent fibres in laminae IV and V of the dorsal horn (Gasser and Graham, 1933; Austin and McCouch, 1955, Beall *et al.*, 1977). Since it is triggered by nonnoxious electrical stimuli, which are known to activate mainly large myelinated fibres, N13 certainly does not reflect the activity of pure nociceptive spinal cells which receive their input from unmyelinated or small myelinated Aδ and C fibres. However, because the neurons generating N13 are close to, or intermingled with, pure nociceptors and wide dynamic range cells in the dorsal horn grey matter (Willis, 1976), we expected an abnormal N13 to be observed in patients with a syrinx in the lower cervical cord and to be correlated with segmental loss of pain and temperature sensation in the territory of the median nerve. Abnormal N13 has been reported in isolated cases of syringomyelia (Emerson and Pedley, 1986; Urasaki, 1988) which lends substance to the above hypothesis.

In this study we report SEP data obtained in 24 patients with syringomyelia showing that the N13 cervical potential is a reliable indicator of the central cervical cord lesion in such patients, while P14 and N20 potentials, presumed to be generated, respectively, in brainstem and parietal cortex, explore selectively the dorsal column/medial lemniscal system dysfunction (Desmedt and Cheron, 1980, 1981*b*; Mauguière and Courjon, 1981; Mauguière *et al.*, 1983*a*; Hashimoto, 1984; Tsuji *et al.*, 1984; Mauguière and Ibañez, 1985; Yamada *et al.*, 1986).

PATIENTS AND METHODS

Patients

Twenty-four patients (10 males, 14 females) aged 20–74 (mean 46) yrs were selected for this study. Clinical and magnetic resonance imaging (MRI) or computerized tomographic (CT) data are given in Table 1. The time interval between onset of symptoms and SEP recording varied between 1 and 35 yrs. All patients had a syringomyelic cavity, documented by MRI in 22 cases and by contrast CT myelography in 2. The interval between MRI (or CT scan) and SEPs was less than 1 yr in 19 patients and less than 6 months in 14. In 4 of the 5 patients for whom this interval exceeded 1 yr (range 2–5 yrs) the diagnosis of syringomyelia had been made long before the SEPs and MRI were examined and all of them had been operated on before recording of the SEPs (Cases 1, 3, 11 and 22). In 1 patient (Case 5) the diagnosis

TABLE 1. CLINICAL DATA

				Somatosensory loss					*Upper limb reflexes*	
				Pain and temperature			*Joint and touch*			
				Side	*Level*		*Upper limb*	*Lower limb*	*Right arm*	*Left arm*
Case	*Sex/age (yrs)*	*Disease duration (yrs)*	*Syrinx level (MRI or CT)*		*R*	*L*				
1	F/57	12	C3-5	B	C5-T1	C6-8	N	N	0	0
2	F/63	15	C3-6*	B	C2-T5	C2-5	B	B	0	0
3	F/43	10	C2-CE	L	–	C4-L2	L	B	0	0
4	M/67	20	C3-8	B	T1-2	C4-T1	N	N	0	0
5	M/44	1	C5-T4	L	–	C2-T5	N	N	N	0
6	M/40	2	C3-T8	L	–	C3-T8	N	N	N	0
7	M/22	1	C3-T3	L	–	C4-T3	N	N	N	N
8	F/42	17	C1-T9	B	C2-T6	C2-T6	L	B	0	0
9	F/52	8	Med-T2	B	C1-6	C7-8	N	N	0	N
10	M/20	1	C3-L2	R	C2-L3	–	N	N	0	N
11	M/46	12	C3-T2	B	C6-T6	C8-T1	R	B	0	0
12	F/44	13	C2-8	R	C5-8	–	N	N	N	N
13	F/58	15	C4-CE	L	–	C2-L5	N	N	N	0
14	M/45	1	C4-T12	R	C2-L2	–	N	N	0	N
15	F/64	35	C2-T1	B	C2-T11	C2-T11	L	B	0	0
16	F/38	3	C2-7	L	–	C2-T5	N	N	N	0
17	F/49	2	C5-7*	B	C2-L2	C7-T1	R	R	0	0
18	F/38	9	C1-T9	L	–	C3-T4	N	B	N	0
19	M/40	7	C3-T1	B	C5-T1	C5-T1	N	N	0	N
20	F/36	6	C1-S1	B	C5-6	C5-T1	N	N	N	0
21	F/51	1	C4-7	L	–	C6-T1	N	N	N	0
22	M/34	3	C3-T9	B	C3-T5	C5-6	N	B	0	N
23	F/74	4	C3-T5	L	–	C5-T1	N	B	N	0
24	M/40	10	C8-T4+ T5-10	L	–	C3-L2	N	N	N	N

* CT scan only; Med = medulla; CE = cauda equina; B = bilateral, L = left; R = right; N = normal; 0 = absent tendon reflexes.

of syringomyelia was confirmed by MRI only 2 yrs after demonstration by SEPs of abnormal cervical responses.

The syringomyelia was associated with an Arnold-Chiari malformation in 16 patients (Cases 1–16). In 9 of the 16 patients with an Arnold-Chiari malformation an occipital craniotomy had been performed before SEP recording completed by a syringoperitoneal shunt in 4 and a syringosubarachnoid shunt in 1. A syringoperitoneal shunt had been performed in 1 of the patients without Arnold-Chiari malformation.

Two patients in whom the diagnosis was made, respectively, 7 months (Case 21) and 8 yrs (Case 22) after a cervical injury were considered as having a posttraumatic syrinx. The latter of these 2 patients had presented a flaccid paraplegia immediately after a fracture with luxation of the T12 vertebra; pain and temperature hypaesthesia of the left upper limb and trunk progressively developed 8 yrs later and prompted MRI investigations; a shunt between the syrinx and the subarachnoid space was performed 2 yrs before SEPs. In the former patient the diagnosis of posttraumatic syringomyelia was not considered to be definite since there had been no vertebral lesion and no symptoms of cord injury immediately after the trauma.

In Case 23 the syrinx was associated with an angioreticuloma at T6 level which was removed surgically 7 months after the SEP recording. Case 24 had been operated for an epidural cyst at T4 level 6 yrs before the diagnosis of syringomyelia was ascertained by MRI. During the interval between surgery and MRI the patient complained of increasing paraesthesiae which were initially confined to the left lower limb then progressed up to involve the abdomen and trunk and finally the whole upper limb of the left side.

In all patients, somatosensory function was carefully analysed at the time of SEP study by testing (1) nonpainful pin prick sensation and touch sensation, the latter quantified by two point discrimination; (2) joint position sense: ability with eyes closed to identify flexion and extension of the fingers and toes at different angular velocities and to reproduce passive joint movements with the control limb; (3) sensitivity to painful pin prick, (4) thermal sensitivity to heat and cold. The type and location of the somatosensory deficit are given for each case in Table 1. Upper limbs tendon jerks were also tested in all patients.

SEP recording methods

For SEP recording, the patient lay on a couch in a warm and semidarkened room. The stimuli (0 2 ms square pulses, with an intensity sufficient to elicit a visible twitch of the thumb) were delivered by skin disc electrodes over the median nerve at the wrist, the cathode attached proximally, interstimulus interval varying randomly between 425 and 525 ms. The bandpass used was 1.6–3200 Hz (–3 dB at cut-off point, 6 dB per octave) with an analysis time of 70 ms (including a 5 ms prestimulus baseline). The bin width was 126 μs. Samples with excess interference were automatically edited out of the average. Two averages of 2000 trials each were obtained and drawn out by the computer on an X-Y plotter.

The recording electrodes (impedance between 5 kΩ) were placed at the supraclavicular fossa (Erb's point), at the spinous process of the C6 vertebra (Cv6), at the contralateral and ipsilateral parietal scalp regions. The Erb's point electrode was referred to Fz and the parietal scalp electrodes to the shoulder contralateral to stimulation.

For the recording of the cervical N13 potential we connected grid 1 of the amplifier to the Cv6 electrode and grid 2 to an electrode located immediately above the process of the thyroid cartilage; this electrode will be referred to in the text and figures as 'anterior cervical' (AC). The rationale for this particular derivation has been explained in the Introduction and exhaustively discussed in previous publications (Desmedt and Cheron, 1981*a*; Mauguière, 1983; Desmedt and Nguyen, 1984; Emerson *et al.*, 1984). In brief, it permits the recording at maximal amplitude of the activity generated by the transverse dipolar source of N13 and tends to cancel the potentials generated above the foramen magnum, as well as the N11 potential, which reflects the ascending volley in the dorsal columns and is picked up by both recording electrodes (Cracco, 1973; Cracco and Cracco, 1976; Desmedt and Cheron, 1980; Anziska and Cracco, 1981; Desmedt and Cheron, 1981*a*; Lueders *et al.*, 1983; Emerson *et al.*, 1984).

This is illustrated in fig. 1A which shows the spatial distribution of the cervical response obtained after stimulation of the right median nerve in a normal subject using a circular array of 8 electrodes disposed around the neck in a plane perpendicular to the neck axis and referred to the shoulder on the nonstimulated side (analysis time: 30 ms, bin width 234 μs). The amplitude of the N13 is maximal at posterior neck (electrode 1.Cv6) and decreases at more lateral electrode sites (electrodes 2 and 8). N13 is absent at electrodes sites equidistant from Cv6 and AC electrodes (electrodes 3 and 7) and reverses its polarity to form a P13 positivity at anterior electrodes 4, 5 and 6. The amplitude of P13 is maximal at the AC site (electrode 5). Right lateral electrodes 6 and 7 on the stimulated side record a triphasic potential preceding the spinal activity which reflects the incoming radicular volley. This triphasic potential, which begins by a P9 positivity, is also present, although attenuated, at electrodes 8 and 5 (AC). The posterior midline Cv6 electrode and all electrodes contralateral to stimulation (2, 3 and 4) record only the P9 positivity but not the following negative-positive deflections; consequently the duration of this P9 potential is longer at these recording sites (fig. 1B). The stationary P9 potential recorded at a distance from the spinal entry zone of stimulated roots reflects the positive front of the afferent volley in cervical roots (Yamada *et al.*, 1980; Desmedt *et al.*, 1983). The trace illustrated in fig. 1C was obtained using a Cv6-AC montage (electrodes 1 and 5 connected, respectively, to grids 1 and 2 of the amplifier). In this trace the P9 positivity picked up by both electrodes is smoothed and often preceded by a small N9 negativity which corresponds to the mirror image of the onset of the anterior cervical P9 injected in the subtraction waveform as a negativity. Thus a small diphasic N9-P9 negative-positive waveform precedes the cervical N13 which is boosted due to injection of anterior P13 positivity in grid 2 of the amplifier. Desmedt and Nguyen (1984) proposed to label '–P9' the negative peak and 'onset of N11' the subsequent positivity that we called P9. This nomenclature was not adopted in this study, where we measured the peak-to-peak amplitudes of the N9-P9 and P9-N13 deflections as illustrated in fig. 1C, since it was possibly confusing. In what follows, the ratio between these two peak-to-peak amplitudes is referred to as the N13/P9 ratio. We have not studied the N11 potential which was never well defined in our control Cv6-AC recordings and absent in 72% of them and thus cannot be considered as a reliable event in Cv6-AC traces (*see above*). In noncephalic reference montage recordings the N11 negativity is picked up with the same polarity at both CV6 and AC recording sites and is superimposed to the posterior N13 and anterior P13 potentials. Thus the N11 shortens the apparent onset latency of the CV6 negativity, while it cancels the initial part of the AC P13 positivity

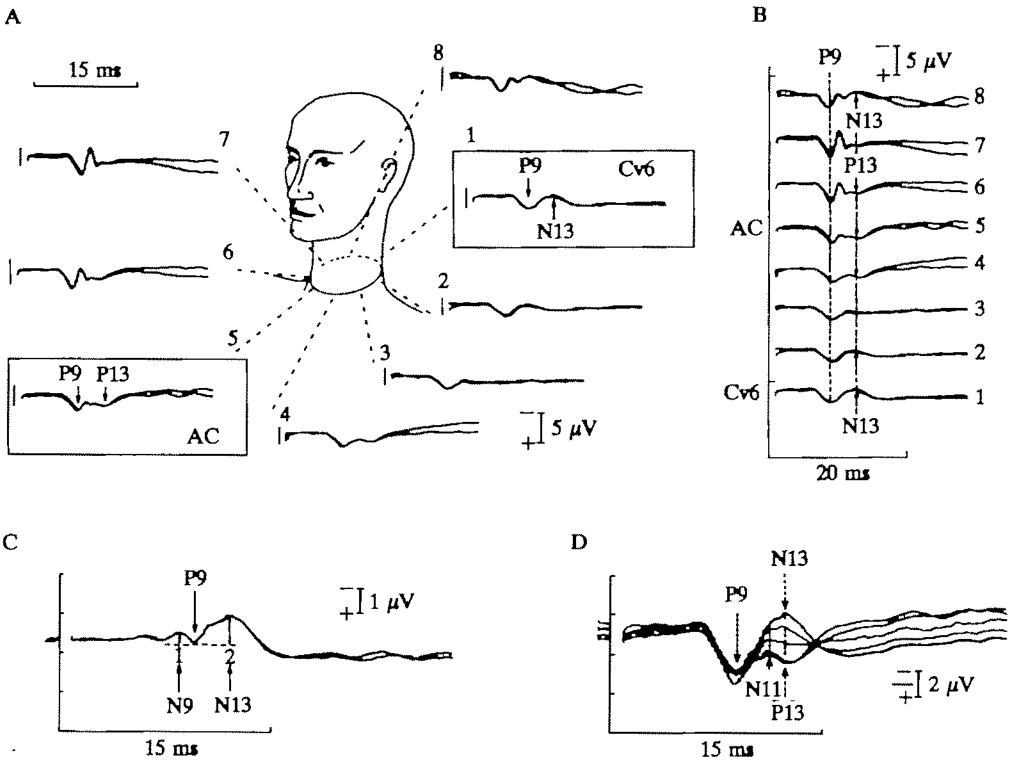


FIG. 1. Normal N13 cervical potential evoked by right median nerve stimulation and recorded using a circular array of cervical electrodes connected to a shoulder reference electrode on the nonstimulated side (A and B). The trace illustrated in C was obtained with the Cv6 anterior cervical derivation In D the superimposition of traces recorded at electrode sites 1–5 (contralateral to the stimulation) shows that the dorsal column N11 is picked up with the same polarity at all recording sites, while there is a polarity reversal of the N13-P13 dorsal horn response.

and causes a shift toward baseline, which artificially increases the onset latency of P13. This overlap in time between the dorsal column N11 and the dorsal horn N13-P13 spinal potentials is illustrated in fig. 1D; it was observed on cord surface and oesophageal recordings (Jeanmonod *et al.*, 1989; Desmedt and Cheron, 1981*a*) and explains why the onset latency of the dorsal horn response cannot be determined accurately.

In 2 patients (Cases 10, 21), the circular array of 8 cervical electrodes illustrated in fig. 1A was used in addition to the standard montage to complete the SEP study.

Normative data (Table 2)

Normative data concerning the N13 potential were obtained from 68 median nerve SEPs recorded in 36 normal subjects (range 15–64, mean 40.4, yrs; 14 men). In 32 both median nerves were studied, in 4 only right median nerve SEPs were available.

For measuring the N13 latency, we considered the interpeak latency N9-N13 and not the absolute latency of N13, in order to eliminate interindividual variations due to arm length differences.

N13 amplitude was measured using the traces recorded with the Cv6-AC montage: (1) from zero volts baseline to peak; (2) between the peaks of N13 and the preceding positivity (figs. 1C, 2), and (3) by calculating the N13/P9 ratio as illustrated in fig. 1C. The distribution histograms of these three parameters in our control population (fig. 2A, B, C) shows that neither the baseline-to-peak nor the peak-to-peak amplitudes had a normal distribution. Moreover, due to well-known difficulties in baseline determination, the low

TABLE 2. NORMATIVE DATA

Normative values for N9-N13 interpeak latency, N13 amplitude and N13/P9 amplitude ratio (36 control subjects)

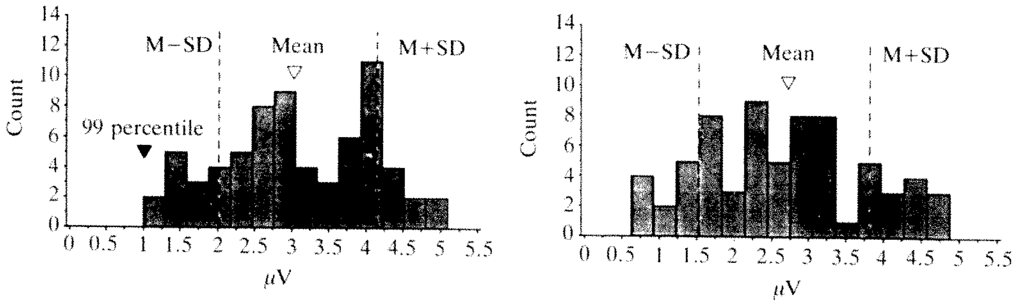
		X	*SD*	*X ± 2.5 SD*	*X ± 3 SD*
Interpeak latency (ms)	N9-N13	3.28	0.4	4.28	4.48
Side-to-side difference (ms)	N9-N13	0.32	0.33	1.14	1.31
N13/P9 amplitude ratio		—	—	1.17	1.02
(*logarithmic values)		(0.395*)	(0.13*)	(0.07*)	(0.005*)
N13 amplitude		(No gaussian distribution; 99 percentile = 1 μV)			

Normative values for P9, P14 and N20 interpeak latencies and P9/P14 amplitude ratio (35 control subjects)

		X	*SD*	*X ± 3 SD*
Interpeak latencies (ms)	P9-P14	4.56	0.52	6.12
	P14-N20	4.87	0.58	6.61
Side-to-side difference (ms)	P9-P14	0.36	0.22	1.02
	P14-N20	0.35	0.32	1.31
P9/P14 amplitude ratio (from P9 onset)		0.65	0.22	1.31

A N13 amplitude (from P9 peak to N13 peak)

B N13 amplitude (baseline to peak)



C N13/P9 ratio

D Logarithmic transformation of N13/P9 ratio

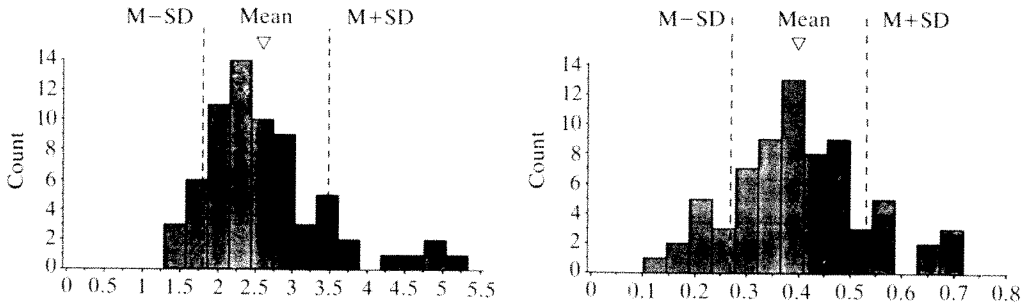


FIG. 2. Histograms of N13 amplitude in controls.

values of baseline to peak N13 amplitudes were more scattered and unevenly distributed than the peak-to-peak values. Although extended to the right by a tail of high values, the distribution of the N13/P9 ratio was closer to a gaussian profile which was achieved after logarithmic transformation of the data (fig. 2D). We therefore adopted as the lower limit of the normal N13/P9 ratio the mean value minus 2.5 SD on the logarithmic scale of fig. 2D. This corresponded to a $\log_{10}$(N13/P9) of 0.07 and to a ratio of 1.17. Moreover, we also considered as abnormal a P9-N13 amplitude lower than 1 μV (99 percentile, fig. 2A).

The rationale for using the N13/P9 amplitude ratio is the test-retest stability of the P9 potential, and the notion that the amplitude of the P9 potential closely reflects the incoming volley in the cervical roots which is likely to be unaffected in spinal cord diseases. Because most of our patients presented with both loss of pain sensation and muscle weakness, in the upper limbs the possibility could not be excluded that the stimulus intensity necessary to provoke a twitch of the thumb could be higher than in control subjects, thus recruiting more high threshold peripheral sensory fibres. It was therefore checked in 4 of our control subjects that the value of the N13/P9 amplitude ratio was not affected by the voltage of the electrical stimulus. We observed that the N13/P9 ratio was not significantly different when tested at motor threshold (mean 2.34; SD 0.31) and at twice the motor threshold (mean 2.12; SD 0.58; *see* fig. 3).

For assessing the conduction in the dorsal column system we measured the P9-P14, P14-N20 interpeak latencies as well as the P9/P14 amplitude ratio. These parameters were measured on the contralateral parietal traces recorded with the shoulder reference montage as described in a previous study (Garcia Larrea and Mauguière, 1988), from which the norms given in Table 2, obtained in a population of 35 subjects, are derived.

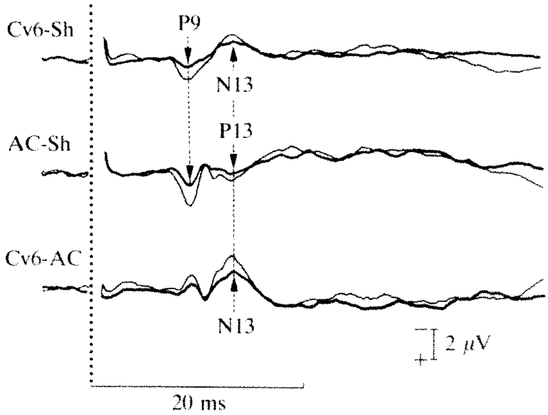


FIG. 3. Amplitude changes of the N13 cervical potential in a normal subject, after stimulation at motor threshold (thick traces) and twice the motor threshold (thin traces). Both P9 and N13 components are increased by raising the stimulation voltage in noncephalic reference recordings (Cv6-shoulder and anterior cervical-shoulder (AC-Sh)), but the N13-P9 ratio in (Cv6-AC) traces is unchanged. Each trace is the average of 4000 stimulations.

RESULTS

SEP group data (Table 3)

The most salient SEP feature was the abolition of the cervical N13 potential that we observed in 32 of the 48 median nerve SEPs (66.7%). Moreover, in 8 responses N13 was persistent but reduced; the N13/P9 ratio was less than 1.17 in all with an absolute N13 amplitude of less than 1 μV in 3. Thus N13 amplitude was abnormal in 40 of 48 median nerve SEPs (83.4%). Conversely, the latency of N13, when measurable, was within normal limits in all individuals, although the mean value of the N9-N13 interval was significantly higher in patients (3.7 ± 0.5 ms, $n = 16$) when compared with normals (Student's t test, $P < 0.01$).

TABLE 3 SEP DATA

	N9-N13 interval		*N13 amplitude*		*P9-P14 interval*		*P14-N20 interval*		*P9/P14 ratio*	
Case	*R*	*L*	*R*	*L*	*R*	*L*	*R*	*L*	*R*	*L*
1	–	–	Abs	Abs	N	N	N	N	N	N
2	–	–	Abs	Abs	–	–	–	–	–	–
3	–	–	Abs	Abs	Abn	–	N	–	Abn	–
4	–	–	Abs	Abs	N	N	N	N	N	N
5	N	N	N	Red	N	N	N	N	N	N
6	N	–	N	Abs	N	N	N	N	N	N
7	N	N	N	N	N	N	N	N	N	N
8	–	–	Abs	Abs	N	–	N	–	N	–
9	–	–	Abs	Abs	N	N	Abn	N	N	N
10	–	N	Abs	N	N	N	N	N	N	N
11	–	–	Abs	Abs	–	Abn	–	N	–	Abn
12	N	N	N	N	N	–	N	–	N	–
13	–	–	Abs	Abs	N	N	N	N	N	N
14	–	N	Abs	N	N	N	N	N	N	N
15	–	–	Abs	Abs	N	Abn	Abn	Abn	N	Abn
16	N	–	Red	Abs	N	N	N	N	N	N
17	–	–	Abs	Abs	–	N	–	N	–	N
18	N	N	Red	Red	N	N	N	N	N	N
19	–	–	Abs	Abs	–	N	–	N	–	N
20	N	–	Red	Abs	N	N	N	N	N	N
21	–	–	Abs	Abs	N	N	N	N	N	N
22	N	N	Red	Red	N	N	N	N	N	N
23	–	–	Abs	Abs	N	N	N	N	N	N
24	N	–	Red	Abs	N	N	N	N	N	N

Abs = absent; Red. = reduced; – = not measurable (N13 or P14 absent), N = normal; Abn = abnormal; R = right side; L = left side.

P14 was the second most affected SEP component. It was either absent or delayed and reduced (prolonged P9-P14 interval and abnormal P9/P14 ratio), respectively, for 8 and 3 median nerves and thus clearly abnormal for 11 of the 48 tested median nerves (22.9%). When measurable the mean P9-P14 interval was not significantly different in patients (4.9±0.9 ms, n = 40) and normals (Student's t test, $P = 0.072$).

The P14-N20 interval was abnormal for only 3 median nerve SEPs; in 2 of them the P14 was normal, suggesting increased conduction time in the intracranial segment of the lemniscal pathway with normal conduction in the dorsal columns. These abnormalities were bilateral in 1 case (15) and unilateral in the other (Case 9). In 7 SEPs the P14-N20 interval was not measurable because both P14 and N20 were absent; for 1 median nerve only the P14 was missing with preserved N20 (Case Nb 12, left side; *see* fig. 9). (The mean value of P14-N20 interval was significantly higher in patients than in normals (Student's t test, $P < 0.01$).)

Clinical correlates of N13 and P14 abnormalities (Table 4)

In Table 4 N13 and P14 data were correlated separately with the segmental sensory loss in the corresponding upper limb. Reduced or absent N13 highly correlated with lost or reduced pain and temperature sensations in the median nerve territory, but not with joint or touch hypaesthesia. The reverse correlation was observed between P14 abnormalities and impaired joint and touch sensation.

TABLE 4 SENSATION, REFLEXES AND N13 P14 SEPs

		Clinical features in corresponding upper limb					
		Pain temperature sensation		*Joint touch sensation*		*Tendon reflexes*	
		Normal	*Reduced*	*Normal*	*Reduced*	*Normal*	*Absent*
N13							
Normal	8	6*	2*	8	0	8*	0*
Abnormal	40	7*	33*	33	7	11*	29*
P14							
Normal	37	11	26	37*	0*	18	19
Absent or reduced and delayed	11	2	9	4*	7*	1	10

* χ^2 significant ($P < 0.01$)

In Table 4 'absent reflexes' refers to the upper limbs for which both the biceps and the brachioradialis reflexes could not be obtained or were clearly reduced when compared with the other side. Thus only clear-cut asymmetries or bilateral abolitions were considered; in patients with absent reflexes on one side only, reflexes, even if weak, were classified as 'normal' on the other side (Cases 6, 17). Absent or reduced tendon reflexes in upper limbs were correlated with N13 but not with P14 abnormalities.

Abnormal SEP subtypes

When combining all parameters listed in Table 2 four SEP subtypes could be identified which are illustrated in figs 4–9.

Type I. Normal SEPs. Normal SEPs were obtained for 7 median nerves (15%); SEPs were normal on both sides in 1 patient (Case 7) only. Normal tendon jerks and joint and touch sensation were constantly associated with this SEP pattern; pain and temperature sensation, however, was impaired in the territory of 2 of the 7 median nerves with normal SEPs. This pattern is illustrated in fig. 4.

Type II. Reduced N13 with normal P14 and N20. This pattern illustrated in fig. 5 was obtained for 8 median nerves (16.7%); it was bilateral in 2 patients (Cases 18, 22). Pain and temperature sensation was most often impaired (5/8) but joint and touch sensation was consistently normal. Reflexes were absent in 3 of the 8 upper limbs with this SEP pattern.

Type III. Absent N13 and normal P14 and N20. This type was definitely the most frequent since observed for 22 median nerves (45.8%); it was bilateral in 6 patients (1, 4, 9, 13, 21, 23). This pattern illustrated in figs 6 and 7 was often associated with reduced pain and temperature sensation (19/22) and absence of tendon jerks (16/22); joint and touch sensation was consistently normal. Absent N13 and normal P14 were associated with an abnormal P14-N20 interval in right median nerve SEPs of Cases 9 and 15. In the former patient, MRI showed that the syrinx extended into the medulla and clinical examination revealed a right palatal palsy and a right hemifacial hypaesthesia. The latter patient had been operated on for an Arnold-Chiari malformation (occipital craniotomy, syringoperitoneal shunt) and had atrophy of the cervical cord and medulla on MRI. In both patients damage to the lemniscal fibres in the lower brainstem or to cuneate nucleus neurons in the medulla is the most likely reason for the increased P14-N20 central conduction time.

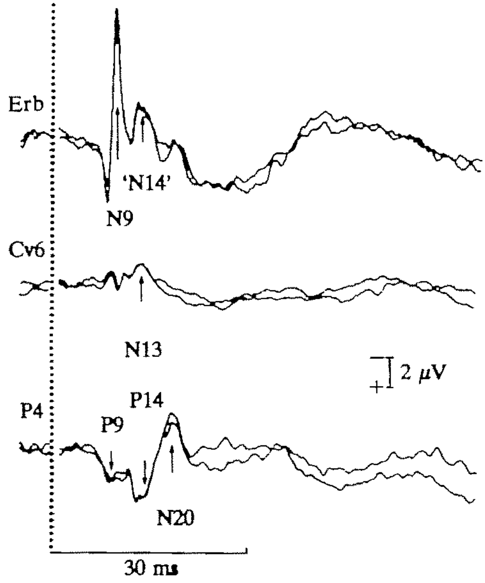


FIG 4 Type I. normal SEPs *Case 10*, left median nerve This patient presented with a segmental loss of pain and temperature sensation (C3–L2) and absent reflexes on the right side. Sensation was normal on the left, as were the SEPs. Two traces obtained on two successive runs of 2000 stimulations are superimposed. Erb = Erb's point connected to a forehead reference electrode (Fz) The P14 potential picked up the frontal electrode is injected as an 'N14' negativity Cv6 = sixth cervical vertebra, connected to an anterior cervical reference electrode P4 = right parietal electrode, connected to the left shoulder (noncephalic reference electrode)

Type IV. Absent N13 with abnormal P14 or N20 (fig. 8). Ten median nerve SEPs belonged to this category (20.8%). This pattern was bilateral in 3 patients (2, 3, 11). In the corresponding upper limbs tendon reflexes were invariably absent; a segmental loss of pain and temperature and of joint and touch sensation was observed respectively in 9 and 7 out of 10.

For 1 median nerve only we observed an unusual SEP pattern consisting of a normal N13 with absent P14 and normal N20 (fig. 9).

DISCUSSION

The major finding of this study is that an abnormal or absent cervical N13 component was observed in 83% of median nerve SEPs in patients with a cervical syrinx, and correlated well with a segmental loss of pain and temperature sensation and absent tendon reflexes in the upper limb. In addition, the scalp P14 and N20 potentials were preserved in 75% of the SEPs with abnormal N13. Up to now, in spite of a few case reports (Mastaglia *et al.*, 1979; Emerson and Pedley, 1986; Kaplan *et al.*, 1988; Urasaki *et al.*, 1988), there was no clear evidence based on group data that this dissociation between cervical N13 and scalp P14 and N20 could be related to any form of dissociated sensory loss.

Since the early study by Halliday and Wakefield (1963) the concept that cortical SEPs are affected only in patients with clinical evidence of dorsal column involvement has been widely accepted. This has been confirmed by several reports of normal scalp SEPs in patients with anterolateral cordotomy and dissociated loss of pain and temperature sensation (Giblin, 1964; Larson *et al.*, 1966; Namerow, 1969). Our data reinforce this view by showing (1) that not only the parietal N20 but also the far-field P14 can be normal after stimulation in the area of pain and temperature segmental anaesthesia in

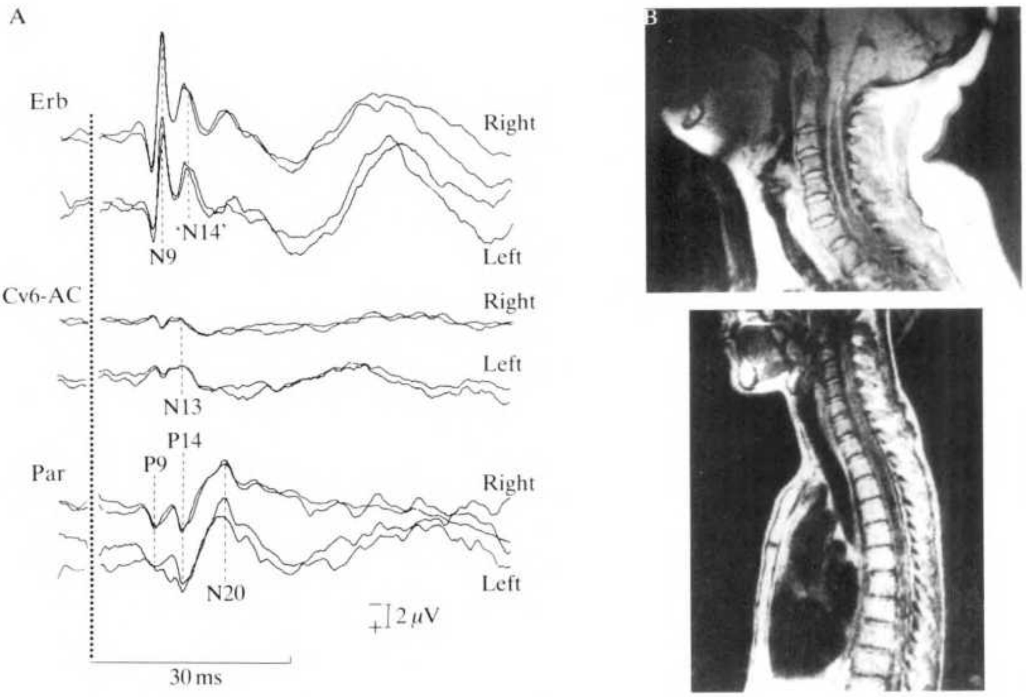


FIG. 5. Abnormal SEPs type II: reduced N13 with normal P14 and N20 potentials. *Case 18.* This patient had an 8 yr history of pain in the left shoulder and arm. Six years after the onset, pain and temperature sensory loss, causing several accidental burns, and hand weakness, which was more pronounced on the left, developed. Two months before the SEP study, a syringoperitoneal shunt had been performed. Tendon jerks were absent in the left upper limb and considered as normal, though possibly reduced, in the right upper limb. A, right and left median nerve SEP. Erb = Erb's point connected to a forehead reference electrode. Cv6-AC sixth cervical vertebral electrode, connected with an anterior cervical electrode. PAR = contralateral parietal electrode (shoulder reference). The N13 potential is present on both sides but it is reduced in amplitude (absolute amplitude: less than 1 μV; N13/P9 ratio less than 1,17), the P14 and N20 potentials are normal in latency and amplitude. B, cervical and thoracic spinal cord MRI (T_1 weighted), showing a syrinx from C1 to T9 with an Arnold-Chiari malformation.

syringomyelia, and (2) that abnormalities of these two components correlate only with lost or impaired joint and touch sensation (Table 4).

Though the notion that the scalp SEPs does not depend on the activity of the spinothalamic tract can be considered as firmly established, it remains to be determined to what extent the postsynaptic activity of cells of origin of the spinothalamic tract contribute to the genesis of cervical N13. Our data do not address directly this question but provide evidence that transmission of pain and temperature inputs is almost constantly impaired when the N13 potential is abnormal.

It may be wondered why earlier studies failed to demonstrate this correlation and reinforced the idea that median nerve SEPs are useless for the functional assessment of the cervical spinal cord in syringomyelia, since they are usually normal in the presence of dissociated loss of pain and temperature sensations (Veilleux and Stevens, 1987). The most likely explanation is the wide use of electrode montages between the posterior neck and a forehead reference electrode (Ganes, 1980; Hume *et al.*, 1982) which precludes a reliable measurement of N13 amplitude. Even when recorded with a noncephalic reference electrode, which does not record the subcortical scalp far-field potentials, the cervical N13 is a complex event in which activities reflecting dorsal column volleys and postsynaptic spinal responses overlap in time. When the posterior neck is connected to the frontal electrode the response waveform includes an 'N14' negativity, which is superimposed on the genuine spinal components and corresponds to the brainstem P14 potential picked up on the scalp (Desmedt and Cheron, 1980; Mauguière and Ibañez, 1985; Emerson and Pedley, 1986). The scalp P14 is far larger than the spinal N13 when these two potentials are recorded, respectively at forehead and at Cv6 process, using a noncephalic reference montage. Consequently the inverted P14 ('N14') is the major contributor to the cervical-to-forehead response in the latency range of N13 (Mauguière, 1989). Since P14 was found to be normal in 30 of the 40 abnormal N13 SEPs in this study, the reduction or absence of N13 would have been overlooked in most of our cases if a frontal reference electrode had been used.

In practice, a noncephalic reference montage which, on theoretical grounds, would be appropriate for assessing the N13 amplitude, is less reliable than the Cv6-AC derivation that was used in this study. N13 is a compound postsynaptic potential and a reduced, or time dispersed, input to dorsal horn cells as a well as reduced number of the dorsal horn cells themselves are likely to decrease the amplitude of the N13 rather than to delay its peaking latency. Indeed the abnormalities of the cervical response that we observed concerned only the amplitude but not the latency of N13. This observation has already been made by Small *et al.* (1978) in patients with multiple sclerosis and abnormal cervical SEPs. With a noncephalic reference electrode montage the N13 amplitude at the posterior neck is very small in normals with a high degree of

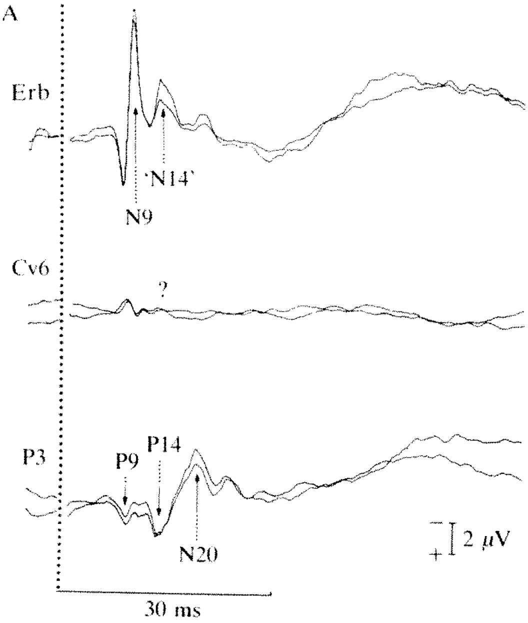


FIG. 6. Abnormal SEPs type III(A): absent N13 with normal P14 and N20. Same patient as in fig. 4. A, right median nerve SEP. Same montage as in fig. 4. The middle trace (Cv6-AC) shows the complete abolition of the N13 potential. The bottom trace from the top (P3 = left parietal, referred to left shoulder) shows normal P14 and N20 potentials. B, cervical cord MRI (T_1 weighted, sagittal midline and transverse planes). The syrinx is clearly lateralized on the right in the spinal segments corresponding to the entry zone of the C6-7 roots.

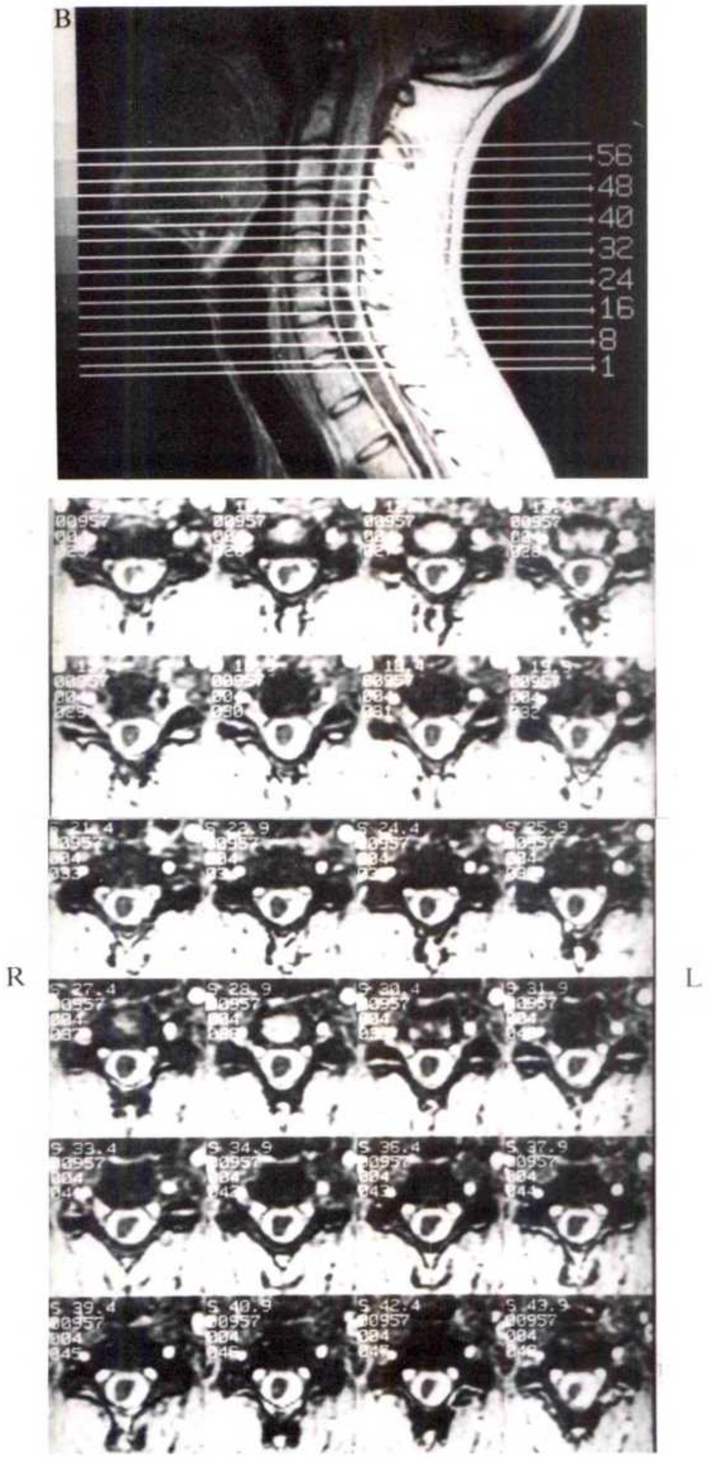


FIG. 6B.

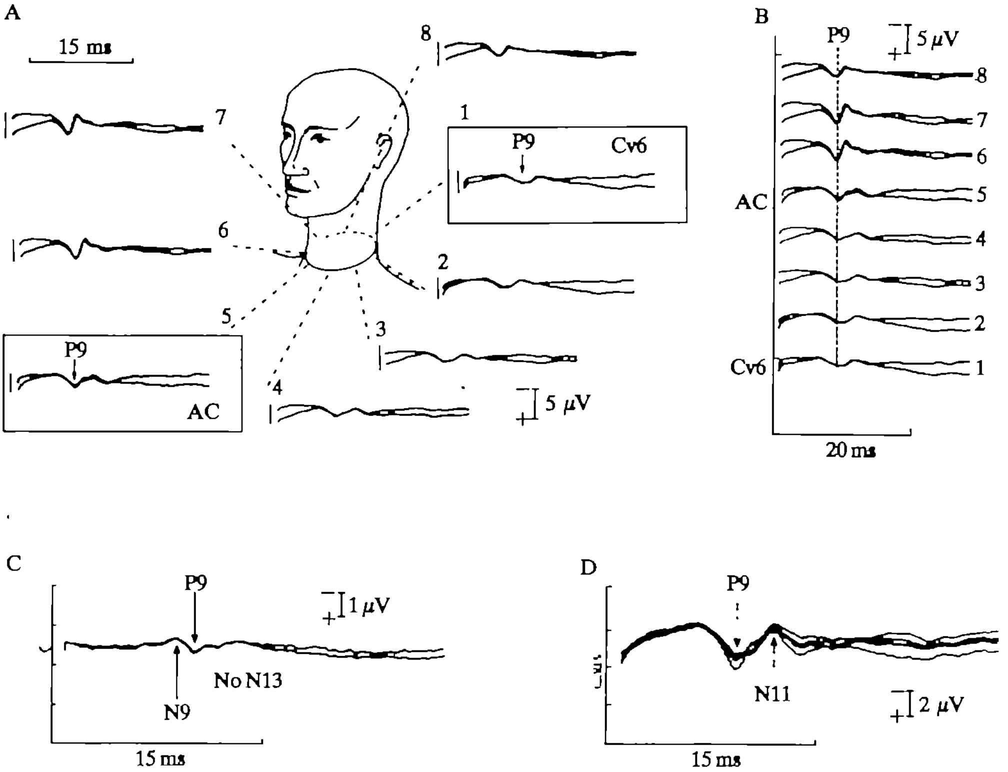


FIG 7. Abnormal SEPs type III. absent N13 with normal P14 and N20 Same patient as in figs 4 and 6, right median nerve SEP; same presentation as in fig. 1 Neither the posterior cervical N13 nor the anterior P13 can be identified (A, B) and the N13 potential is clearly absent in Cv6-AC trace (C) In D the superimposition of traces 1 to 5 shows the persisting N11 posterior column response, with no polarity reversal between anterior and posterior neck recording sites, and the absence of the N13 and P13 potentials. These traces should be compared with those illustrated in fig 1D showing the normal waveform when both the N11 and the N13-P13 potentials are present and overlapping in time.

interindividual variability (0.47 ± 0.35 μV in Desmedt and Cheron, 1981*a*). This hampers the gathering of a reliable normative data bank of N13 amplitudes, which could be achieved in this study by using a montage picking up the activity generated by the horizontal dipolar source of N13 with its maximal amplitude.

An alternative to the Cv6-AC montage would be the routine use of a circular array of electrodes around the neck referred to a noncephalic electrode. A selective loss of the Cv6 N13 and of the anterior cervical P13 could thus be evidenced by Emerson and Pedley (1986), Urasaki *et al.* (1988) and ourselves, respectively, in 3, 1 and 2 patients with syringomyelia. Surface recording of potentials generated by nuclear structures depends on the orientation of the cellular elements with respect to the recording electrodes (Lorente de Nó, 1947). Therefore, the rationale for this circular montage is that it could differentiate between a change in the orientation of the N13 dipolar source due to a distortion of the spinal cord and a reduced or absent response of spinal neurons. Nevertheless, in none of the reported patients to date is there any evidence that this

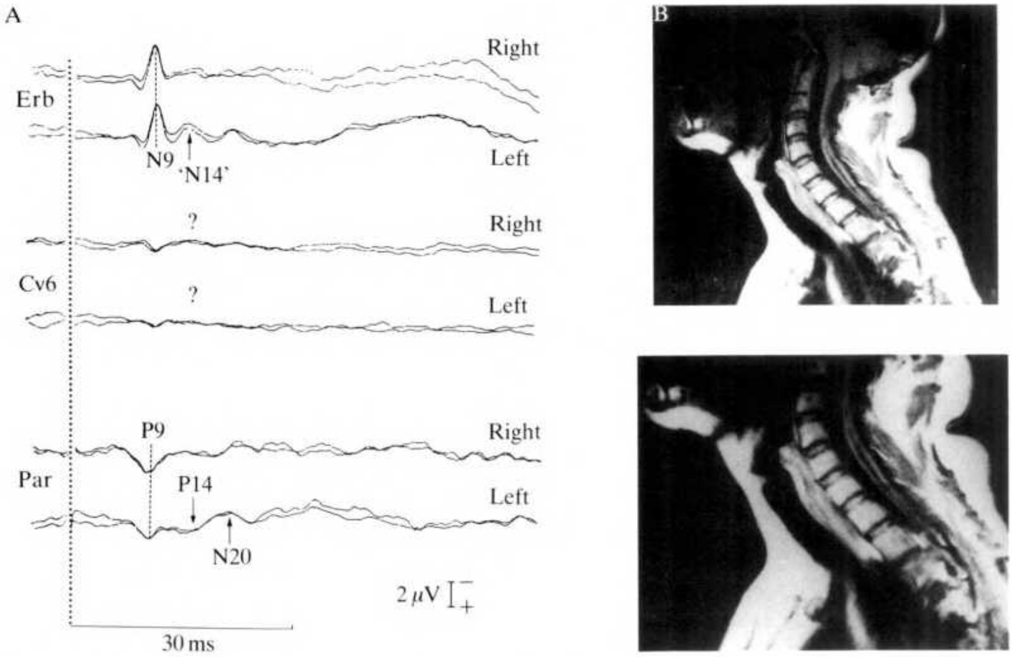


FIG. 8. Type IV SEPs: absent N13, abnormal P14. *Case 11.* This patient had complained for 12 yrs of progressive pain and temperature sensory loss, initially confined to the right upper limb. One yr after onset, a spinal cord CT scan showed an Arnold-Chiari malformation. He had been operated on twice (occipital craniotomy 1 yr after onset, syringoperitoneal shunt 3 yrs after onset). At the time of the SEP recording, he presented with a gait disturbance, complete sensory loss in the lower limbs and right upper limb, a dissociated loss of pain and temperature sensation in the left upper limb, and complete loss of tendon jerks in both upper limbs. A, right and left median nerve SEPs. Erb = Erb's point connected with forehead reference electrode. For the right median nerve the 'N14' potential, corresponding to the P14 scalp far-field, is absent. Cv6 = cervical response, recorded using a Cv6 anterior cervical montage. The N13 potential is bilaterally absent. Par = parietal electrodes contralateral to stimulation, referred to the shoulder contralateral to stimulation. Both P14 and N20 are absent on stimulation of the right median nerve; stimulation of the left median nerve evoked a delayed P14 of reduced amplitude and an N20 potential B, cervical cord MRI (T_1 weighted) showing a cervical syrinx with an associated Arnold-Chiari malformation.

sophisticated montage reveals an abnormality of N13 related only to a rotation of the dipolar source in the horizontal plane, or that it could detect an abnormal N13 which could have remained unnoticed with the Cv6-AC derivation.

The finding that a segmental loss of pain and temperature sensation was present and reflexes absent in the stimulated area for 82.5% and 72.5%, respectively, of the N13 abnormal SEPs (Table 4) constitutes positive proof that a mere distortion of the cord was not the underlying mechanism of abnormal SEPs in any of these cases. Moreover, in 3 patients (6, 10, 14) both the N13 abnormality and the pain and temperature sensory loss were strictly lateralized; this strongly suggests that a mere distortion of the spinal cord is not sufficient to cancel the cervical response after stimulation of the unaffected side (fig. 7).

In 7 patients (3, 13, 16, 18, 21, 23, 24) we found a bilateral abnormality of N13, whereas the segmental and dissociated loss of pain and temperature sensations was

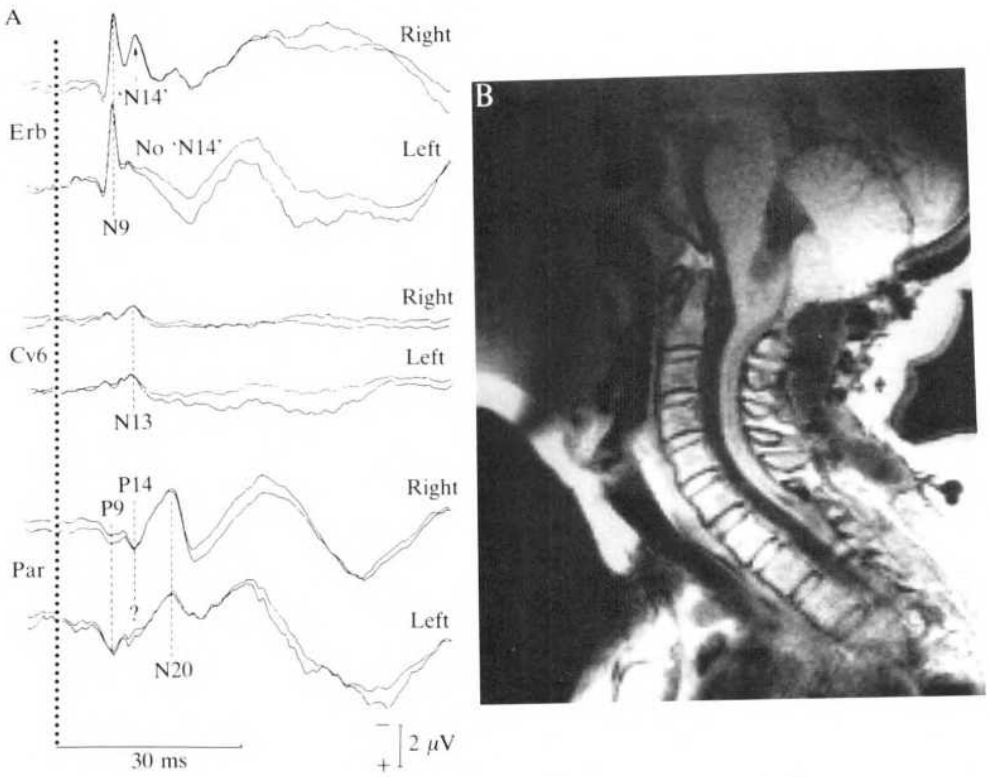


FIG. 9. Reduced P14 with normal N13 and N20 potentials. *Case 12.* This patient had complained of severe headaches for the 12 yrs preceding the SEP study: 1 yr after the onset, the diagnosis of an Arnold-Chiari malformation was made, on the basis of clinical features (left XI and XII cranial nerve palsies, central vertigo, impaired temperature sensation in the C6 and C7 territories on the right) and myelographic study. The patient underwent two surgical operations (occipital craniotomy and syringoperitoneal shunt). At the time of the SEP study, the clinical examination showed instability in standing, with a tendency to fall towards the right, and reduced pain and temperature sensation in the C5–8 territory on the right side. The upper limb tendon jerks were only slightly reduced on the right side. A, right and left median nerve SEPs, same abbreviations as in fig. 8. The only abnormality is the absence of the P14 potential after stimulation of the left median nerve. Note that in the Erb's point to Fz trace 'N14' is absent for stimulation of the left median nerve. B, cervical cord MRI (T_1 weighted) revealing a syrinx extending from C2 to C8. The abolition of the P14 potential can be explained by increased temporal dispersion of conduction in the lemniscal pathways.

unilateral. Absent N13 after stimulation of an upper limb which does not show any clinically obvious sensory loss could result from (1) a subtle pain and temperature hypaesthesia that remained unnoticed on clinical examination because sensation was clearly lost on the other side; (2) an anteriorly developed syrinx, respecting the more dorsal neurons but reducing the postsynaptic activity of cells receiving inputs from Ia afferents (in fact, clinical examination in Cases 3, 13, 18 and 21 revealed signs of muscle weakness and wasting in both upper limbs); (3) a high sensitivity of N13 to a partial neuronal loss of dorsal horn cells which does not cause clinically detectable hypaesthesia. Clinical examination of pain and temperature sensation may fail to detect hypaesthesia, when the impairment is bilateral but asymmetric, since hypaesthesia may be overlooked on the less affected side. In that respect, the percentage of patients in whom the segmental loss of pain and temperature sensation was unilateral in the C6-7 territory was high (54%), but it was substantially similar to that reported by Schliep (1978) in a series of 107 patients (50/107; 46.7%).

In some of our patients with unilateral segmental sensory loss in C6-7 territory, hypaesthesia was indeed bilateral for the upper cervical dermatomes, suggesting that, in the lower cervical cord, the syrinx damaged the dorsal horn on one side only and spared most of the spinothalamic fibres which cross the midline at this level. Follow-up studies of patients with syringomyelia have shown that the moment when clinical signs of posterior column involvement become apparent represents a significant downward step in the natural history of the disease. Disturbances of joint and touch sensation usually occur later on in the progression of the disease (Schliep, 1978); SEP evidence of impaired conduction in the posterior columns therefore represents an important marker for progression of the disease. Our data show that the P14 potential is a reliable index of posterior column dysfunction: joint and touch sensation was never abnormal without a concomitant abnormality of the P14 potential; conversely, it was normal in 4 patients with abnormal P14 SEPs. This confirms the sensitivity of the P14 potential in disclosing subclinical impairment of the posterior column system previously demonstrated in patients with discrete cervicomedullary lesions or multiple sclerosis (Mauguière and Ibañez, 1985; Yamada *et al.*, 1986; Garcia Larrea and Mauguière, 1988).

The mean P14-N20 interval was significantly greater in patients than in normals. This finding is clearly independent of spinal cord damage, since increased P14-N20 conduction time reflects a slowing of conduction in the intracranial segment of the somatosensory pathways. Cerebellar ectopia with stretching and probably direct compression of the medulla is the most likely reason for these group differences between patients and normals since an Arnold-Chiari malformation was present in 16 of our 24 patients.

The four SEPs subtypes identified in this study are closely related to the stage of evolution of the disease, and allows assessment of both the segmental as well as posterior column dysfunction. Due to their high degree of sensitivity in syringomyelia SEPs can be recommended as a routine means of investigating cervical cord function and in guiding neuroimaging and neurosurgical decisions.

ACKNOWLEDGEMENTS

The authors are indebted to Dr Martin Halliday for having reviewed the manuscript and to all our colleagues who referred their patients to our department for SEP recordings and thus helped in assembling the patient material.

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(Received November 8, 1989. Revised March 6, 1990. Accepted March 9, 1990)

Brain (1991), **114**, 381–403

CONGENITAL MIRROR MOVEMENTS

ABNORMAL ORGANIZATION OF MOTOR PATHWAYS IN TWO PATIENTS

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SUMMARY

We studied 2 patients with congenital mirror movements by means of various neurophysiological and metabolic techniques, including mapping of motor evoked potentials to transcranial electrical and magnetic stimulation, premovement and somatosensory evoked potentials, kinematics of voluntary movements, muscle reflexes, and positron emission tomography (PET). Abnormalities in maps of motor and premovement potentials and in PET scans were consistent with a bilateral representation of hand muscles in the motor cortex, the existence of physiologically active connections capable of conducting fast efferent volleys from the motor cortex to ipsilateral muscles, the presence of mirror EMG activity in either hand with intended voluntary movement of the other hand, the absence of mirrored EMG responses from wrist flexors and extensors to mechanical perturbation of the contralateral wrist, and normal scalp distribution of somatosensory evoked potentials to right and left median nerve stimulation. Our findings are consistent with aberrant organization of motor representation areas and corticospinal pathways with ipsilateral as well as contralateral control of voluntary movement.

INTRODUCTION

Mirror movements are involuntary movements on one side of the body that occur as mirror reversals of an intended movement on the other side of the body. These movements were described by Erlenmeyer as early as 1879. They are common as normal phenomena in young children and usually disappear after the first decade of life (Connolly and Stratton, 1968), coinciding with the completion of myelination of the corpus callosum (Yakovlev and Lecours, 1967). When mirror movements persist after that age, they are abnormal and may be familial (Guttmann *et al.*, 1939; Cohn and Kurland, 1958; Haerer and Currier, 1966; Regli *et al.*, 1967; Somers *et al.*, 1976; Schott and Wyke, 1981) or sporadic (Baird *et al.*, 1967; Gunderson and Solitare, 1968; Gardner, 1979; Schott and Wyke, 1981). Mirror movements have been described in association with hypogonadotrophic hypogonadism and anosmia as part of Kallmann's syndrome (Kallmann *et al.*, 1944; Conrad *et al.*, 1978; Schwankhaus *et al.*, 1989), and also in association with Klippel-Feil syndrome (Bauman, 1932; Baird *et al.*, 1967; Gunderson and Solitare, 1968; Gardner, 1979; Schott and Wyke, 1981), Usher's syndrome (Forget *et al.*, 1986), Parkinson's disease (Guttmann *et al.*, 1939), Friedreich's ataxia (Regli

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et al., 1967), Arnold-Chiari malformation (Schott and Wyke, 1981), phenylketonuria (Friedman and Levinson, 1954), behavioural deficits (Woods and Eby, 1982) and pyramidal tract defects (Walshe, 1923; Berlin, 1951). When they are present in families without other neurological involvement, the inheritance is usually autosomal dominant with incomplete penetrance (Meyer, 1942; Johnston, 1948; Haerer and Currier, 1966). Despite the relative similarity of its clinical features, this syndrome is relatively heterogeneous (Schott and Wyke, 1981).

True mirror movements should be differentiated from associated movements observed in patients with hemiparesis (Marie and Foix, 1916; Zülch and Müller, 1969; Ausman *et al.*, 1978), stroke, brain tumours (Roski *et al.*, 1982), intracerebral or subarachnoid haemorrhage (Hopkins *et al.*, 1982; Caplan, 1983), ataxia (Miyamoto *et al.*, 1984) and spinal cord lesions (Levin and Margolis, 1977; Barnett *et al.*, 1978). In hemiplegics, onset of movement in the paralytic spastic limb is delayed in comparison wtih the normal limb (100–300 ms) (Walshe, 1923; Green, 1967; Hopf *et al.*, 1974; Forget *et al.*, 1986), effort is usually required to trigger the phenomenon (Marie and Foix, 1916; Zülch and Müller, 1969; Hopf *et al.*, 1974), and the associated movement occurs only in spastic limbs (Guttmann *et al.*, 1939). In patients with congenital mirror movements, onset of EMG activity may be nearly simultaneous in both limbs (Forget *et al.*, 1986). Such patients do not have spasticity (Guttmann *et al.*, 1939).

The mechanism for production of mirror movements is unknown. To help to elucidate the neurophysiology of this phenomenon, we thoroughly studied 2 patients with mirror movements. Among a variety of studies, we investigated the output connectivity of motor representation areas by mapping motor evoked potentials elicited by transcranial electrical stimulation (Merton and Morton, 1980; Marsden *et al.*, 1982; Merton *et al.*, 1982; Levy *et al.*, 1984; Hassan *et al.*, 1985; Rossini *et al.*, 1985; Day *et al.*, 1987; Rothwell *et al.*, 1987; Cohen and Hallett, 1988*a*, *b*) and by mapping premovement potentials (Shibasaki *et al.*, 1980; Neshige *et al.*, 1988). Because passive finger movements may trigger contralateral finger movements in affected patients (Drinkwater, 1914; Guttmann *et al.*, 1939; Schott and Wyke, 1981), suggesting an abnormality of sensory representation areas with bilateral projection of afferents, we also studied stretch reflexes and multichannel somatosensory evoked potentials, a technique used for noninvasive mapping of human somatosensory cortex (Allison *et al.*, 1980; Duff, 1980; Desmedt and Bourguet, 1985).

PATIENTS AND METHODS

Case 1

The patient, a 28-yr-old man, did not show unilateral hand and leg movements when a baby. His parents stated that he never crawled because both legs always moved simultaneously. He started to walk late, and balance was a problem during his infancy. Even simple manual tasks were done with both hands. He could play a guitar as long as his right hand did not perform independent finger movements. He could swim using the breast stroke, but could never master the crawl. He could not climb a rope or ladder or perform percussion during medical school years. Until his high school years, running was impossible because of lack of alternating arm movements. The patient's personal and family history is negative for any other movement disorders. His puberty occurred at 12 yrs of age. He has a scoliosis, but has no endocrine, olfactory or taste problems. Neurological examination revealed mirror movements in both upper extremities, but not of the legs or face. No other neurological abnormalities were detected. Magnetic resonance imaging (MRI) of the head revealed no abnormalities.

Case 2

The patient, a 38-yr-old woman, has had mirror movements since infancy. Her parents believed her difficulties increased with the need for more precise and unilateral tasks as she matured. These difficulties were characterized by teachers and physicians as 'delay in motor development'. Very simple unilateral skills had to be learned over several years. For example, it took her 4 yrs to master the crawl swimming technique and to be able to move one arm at a time. She could swim using the breast stroke, since it did not require independent arm movements. Her birth was premature. Her medical history included a ruptured ovarian cyst, episodes of migraine, and episodes of depression with suicide attempts on 3 occasions; the depression was treated with drugs, but not with electroconvulsive therapy. The patient's family history is positive for mirror movements in her 2 children (aged 6 and 9 yrs), who also have school difficulties, and in her father's mother. Neurological examination showed mirror movements in both upper extremities, but not of the legs or face. Movements capable of triggering mirror responses were writing, typing, opening and closing one hand in a grip, and tapping her knee with one hand. Smell and taste were preserved and no other neurological abnormalities were found. MRI (0.5 tesla) of the head revealed no abnormalities.

Motor evoked potentials

The technique used for recording and mapping motor evoked potentials (MEPs) elicited by electrical stimulation has been described elsewhere (Cohen and Hallett, 1988*a*). It involves the delivery of constant voltage stimuli through a bipolar surface stimulator (2.5 cm between anode and cathode) with a Digitimer D-180 while the subject is at rest. The anode was positioned over the desired scalp location and the cathode 2.5 cm anterior to the anode. Low impedance was maintained between anode and cathode. The stimulus intensity was increased over the theoretical upper or lower limb motor representation areas until a small muscle response was achieved. The same stimuli were delivered over the different scalp locations corresponding to the same limb representation area (i.e., hand). Locations stimulated were less than 2.5 cm apart in order to increase spatial resolution. We only stimulated scalp positions which either evoked or were immediately adjacent to those which evoked MEPs from the muscles studied. Three muscle responses were recorded for each location to assess the reproducibility of the amplitude and latency of the motor potential. The EMG was amplified and filtered using a bandpass of 100–3000 Hz. The 3 amplitudes were averaged, and the resultant value was assigned to represent that scalp location. When the stimulus did not evoke a muscle response for a certain position, that position was assigned a zero value. The same procedure was then repeated for mapping the motor representation of each body part. Recordings were from surface electrodes positioned on the skin overlying the right and left thenar muscles (TM) and tibialis anterior (TA) during rest. Eight normal volunteers were also studied for comparison with the patients. Background EMG activity was monitored by sound and by looking at the baseline of recorded MEPs.

We constructed colour maps of the MEPs by quantifying the amplitudes of M responses (recorded from thenar muscles with stimulation of different scalp locations) relative to the maximum M response obtained from that muscle to transcranial electrical stimulation.

Conduction time from brain to muscles

To measure conduction time from the brain to muscles, we studied MEPs elicited by transcranial electrical and magnetic stimulation while the subjects performed mild muscle activation. Electrical stimulation was delivered from a Digitimer D-180 through a bipolar montage, with the anode over C3 or C4 and the cathode 8 cm anterior to the anode (Merton and Morton, 1980). Stimulus intensities were those necessary to evoke supramaximal responses (to scalp stimulation) during mild muscle activation and consequently evoked muscle responses at shortest latencies. Magnetic stimulation was delivered from a Cadwell MES-10 stimulator through a 9.5 cm diameter magnetic coil. The inner edge of the circular coil was positioned overlying C3 or C4 with the handle posterior and the coil flat on one side of the scalp. Stimuli were delivered during mild muscle activation of TM and TA (10–30% of maximal muscle contraction) to ensure earliest latencies. The magnetic stimulus intensity was the maximal (100%) delivered by the stimulator (2.2 tesla), which was equivalent to 2 and 1.6 times motor threshold, respectively, when the patients were at rest. Eight normal volunteers were studied for comparison with the patients.

Premovement potentials

We studied the cortical topography of premovement potentials occurring less than 150 ms before movement onset in our patients (Shibasaki *et al.*, 1980; Boschert and Deecke, 1986). The patients sat in an armchair with their elbows slightly flexed and their forearms resting on the armrests while they looked at a target in front of them. They were instructed not to blink and to avoid any facial movements during the recording. They were asked to make self-paced voluntary abductions of the index finger, first with the right hand and then with the left hand. Each finger movement was repeated 150 times. We recorded 29 channels of the EEG, 1 channel of the electro-oculogram, and 2 channels of the electromyogram from the first dorsal interosseous muscle of the right and left hand. The EEG montage consisted of electrodes positioned according to the 10-20 international system of scalp electrode placement plus 2 intermediate rows of 10 electrodes, 5 positioned anterior and 5 posterior to the critical coronal line crossing T3-Cz-T4, referenced to linked earlobe electrodes. The additional electrodes were named according to the midpoint between the 10-20 system electrodes (e.g., C3-P3) The EEG was amplified with a gain of 50 000, using a bandpass of 0.1 – 100 Hz. The rectified EMG of the voluntarily activated muscle triggered the backwards data collection for a window of 499 ms. Later, the individual recordings were realigned precisely according to the start of the EMG (Barrett *et al.*, 1985). Results obtained from patients were compared with those obtained in 14 right-handed normal volunteers (Tarkka and Hallett, 1990).

Particular attention was paid to three components of the potentials preceding movement: negative slope (NS′), premovement positivity (PMP) and motor potential (MP). We measured the part of the MP that we called the initial slope, which was defined as the most focal expression of the highest 21% of negativity occurring after PMP (Tarkka and Hallett, 1990). Peak latencies and amplitudes were measured and topographic maps (*see below*) were created from the averaged waveforms of each individual recording.

Somatosensory evoked potentials

For the study of somatosensory evoked potentials (SEPs), percutaneous electrical stimuli were delivered through bipolar surface electrodes to the median nerve at the right and left wrists of both patients and to the tibial nerve immediately behind the medial malleolus on the right and left lower limbs of Case 1. Stimulus intensity was adjusted to elicit a visible twitch of the thenar muscles when stimulating the median nerve and of the plantar flexors when stimulating the tibial nerve. For each patient, 2000 trials were averaged, and each average was replicated to assess reproducibility. The recording montage was the same as described for premovement potentials. Electrode impedances were below 5 kΩ. SEPs were amplified with a gain of 200 000 using a bandpass of 30 – 1000 Hz and a sampling rate of 4000 Hz.

We constructed colour maps of premovement potentials and SEPs by displaying the amplitudes of these potentials on a 22-colour scale. Black and red represented the maximal negativities and blue the maximal positivities. Values at intermediate pixels of the motor, premovement and somatosensory map images were computed using a second order interpolation algorithm.

Voluntary movements and stretch reflexes

The patients sat in an armchair with both forearms resting on the armrests. Both hands were restrained within a handle that was attached to a torque motor that allowed flexion and extension movements about the wrist. The patient was unable to see his or her hand, but viewed a video screen on which appeared a target cursor (experimenter-controlled) and a hand-position cursor (patient-controlled). Voluntary movements and reflex responses were evaluated in both wrists. For voluntary movements, the patient was instructed to align the position cursor with the target cursor at 10° of flexion for 1.5 – 2.5 s. When the target jumped to a new location on the video screen, corresponding to 30° of flexion, the patient was required to realign the two cursors by flexing the wrist as fast as possible. Each patient performed 25 of these trials. In separate experiments, 2 other voluntary movements were analysed: unilateral voluntary extension of digit III (extensor digitorum communis, EDC III) and abduction of digit II (first dorsal interosseous, FDI).

Reflex responses in the wrist flexor muscles were evaluated by having the position-servo controlled motor smoothly extend the hand 20° in 100 ms. Reflexes were elicited 25 times.

For voluntary and reflex responses about the wrist, a constant load of 0.32 Nm opposed wrist flexion. Surface EMG was recorded from wrist flexor and extensor muscles in both forearms and from the skin overlying EDC III and FDI. The signals were amplified, rectified, low-pass filtered (50 Hz) and then digitized at 200 Hz. Data on hand position were also digitized and stored. Muscle activity responses were quantified by integration with respect to the duration of individual responses. For analysis of data from voluntary movement about the wrist, the hand position and EMG records were centred with respect to the onset of muscle activity from the active hand. For reflex data, the records were centred on the onset of the perturbing ramp. More detailed experimental arrangements are discussed elsewhere (Sanes, 1986). Five normal subjects were also studied for comparison with the patients.

Positron emission tomography

Regional cerebral blood flow was investigated using the NIH Scanditronix Model 1024-7B PET scanner with an in-plane resolution of 5 mm (full width, half maximum), and Z axis resolution of 13 mm (Daube-Witherspoon *et al.*, 1987). A rigid thermoplastic face mask was moulded and used to maintain a fixed head position during the study. Seven slices were obtained parallel to the canthomeatal line and were arranged so that electrodes C3 and C4 of the 10-20 system were near the middle of the penultimate slice. Correction time for skull attenuation was made by the use of a transmission scan once the patient was positioned. In order to allow multiple scans, we injected 30-mCi doses of H_2O^{15} into an antecubital vein. Because we did not sample the radioactivity in the blood, the scan results are expressed in tissue counts and not in absolute blood flow. Scanning time of 1 min gave optimal image quality (Mazziotta *et al.*, 1985) and reduced scatter. The high sensitivity of the scanner allowed the acquisition of approximately 800 000 counts/slice during the 1 min scans. Two scans were obtained with the patients at rest, and 2 were done while the patient performed a motor task consisting of left wrist flexion and extension at about 2 Hz, which was started 30 s before the injection of the tracer and continued for the duration of the scan.

Region of interest (ROI) analysis was performed with the NIH MIRAGe software package. A circle 24 mm in diameter was optimally placed on the motor cortex contralateral to the intended movement in such a way as to obtain a maximal value and at the symmetric anteroposterior position on the ipsilateral cortex, and the mean pixel value was computed. The positions used were identical for the 2 active scans and the 2 rest scans The motor cortex ROI values were normalized by dividing them by the mean pixel value for the entire slice, obtained by placement of single large ROI on the entire slice For both conditions (rest and movement), the 2 values for the contralateral cortex and the 2 values for the ipsilateral cortex were averaged. For both the contralateral cortex and the ipsilateral cortex, the change in tissue activity due to the movement was obtained by subtracting the mean rest value from the mean movement value. Then a ratio of the increment of the ipsilateral to contralateral cortical tissue counts was computed. A value of 1 would indicate identical increments in ipsilateral and contralateral cortices; a value of 0 would indicate no increment in the ipsilateral cortex. The patients' values were compared with those of a control group of 11 normal subjects, 3 men and 8 women, aged 28–69 yrs.

RESULTS

Motor evoked potentials

Unilateral scalp stimulation failed to evoke ipsilateral muscle responses from TM in a group of normal volunteers. Our normal subjects have shown that the hand motor representation area is well localized, always contralateral, and less than 2.3 cm away from the contralateral C3 or C4 (Cohen and Hallett, 1988*a*).

The maximal responses from the right TM of Case 1 were recorded when stimulating on either side of the scalp over C3 (400 μV) and C4 (415 μV) and 2.3 cm behind and medial to C4 (400 μV). Also, responses from the right TM were recorded when stimulating over the midline (*see* 2.3 cm behind Cz in fig. 1). The latencies of EMG responses from the right TM were similar with stimulation of the right and left sides of the scalp (±2 ms).

Fig. 2 illustrates the striking differences between the right-hand motor maps in a normal volunteer and in Case 1. The maximal responses from the left TM of Case 1 were recorded when stimulating ipsilaterally 4.6 cm to the left of Cz, and 2.3 cm to the left of and behind Cz (600 μV in both positions) (fig. 3). Also large responses from the left TM of Case 1 were recorded when stimulating the midline 2.3 cm behind Cz (550 μV) and even the ipsilateral scalp 2.3 cm medial to C4 (300 μV). The latencies of EMG responses

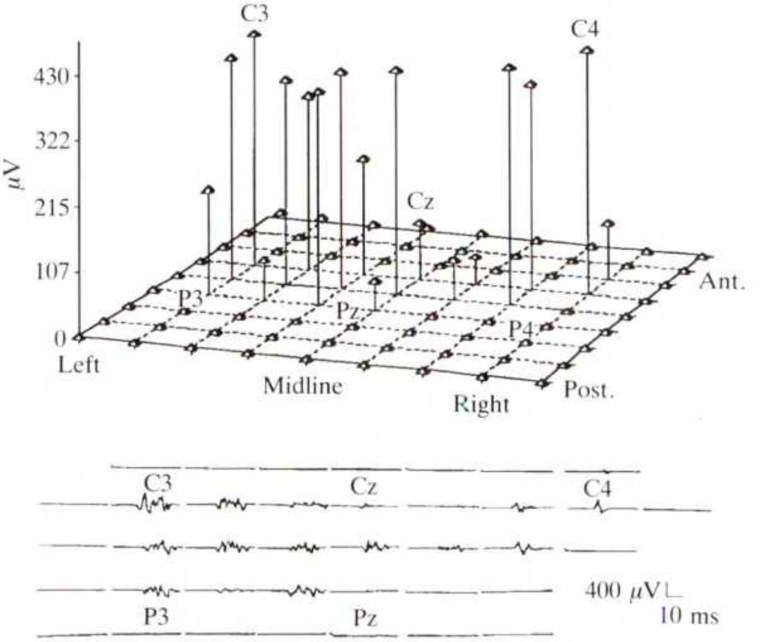

FIG. 1. Right TM motor map in *Case 1* obtained by stimulating different scalp locations with the same stimulus duration and intensity (100 µs, 700 V) using the bipolar short-distance montage (*see* Methods for details). The diagram represents the top of the head. Pyramids show the scalp positions where the stimulating electrodes (anodes) were placed. The height of each triangle shows the mean amplitude (in µV) of 3 muscle responses recorded from the right TM with stimulation of each scalp position. Positions labelled are those coinciding with the 10-20 international system of electrode placement. EMG traces are examples of muscle responses recorded on stimulation of each scalp position. Note the similar latency and amplitude of EMG responses evoked by stimulation of either side of the scalp and the presence of relatively large EMG responses evoked by stimulation of the midline.

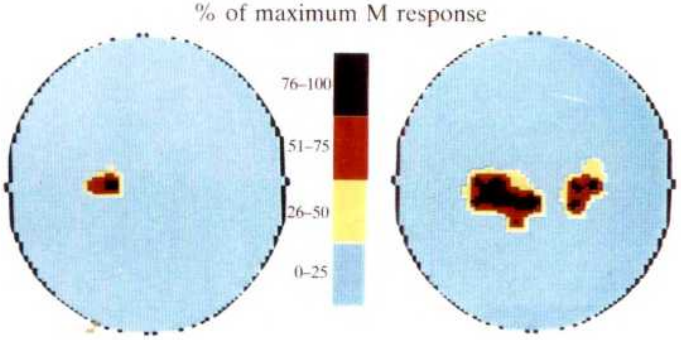

FIG. 2. Right TM motor maps recorded from a normal volunteer (*left*) and from *Case 1* (*right*). The diagrams are views of the top of the head. Black areas show the scalp positions which, on being stimulated, evoked maximal responses from the right TM to transcranial stimulation. Red, yellow, and blue areas show the scalp positions which, on being stimulated, evoked muscle responses of 51 – 75%, 26 – 50% and 0 – 25% of maximal M responses. Blue areas show the scalp positions which, on being stimulated, evoked muscle responses smaller than 25% of the maximal. Note that the map for *Case 1* is larger, poorly localized, and bilaterally distributed in relation to that of the normal volunteer.

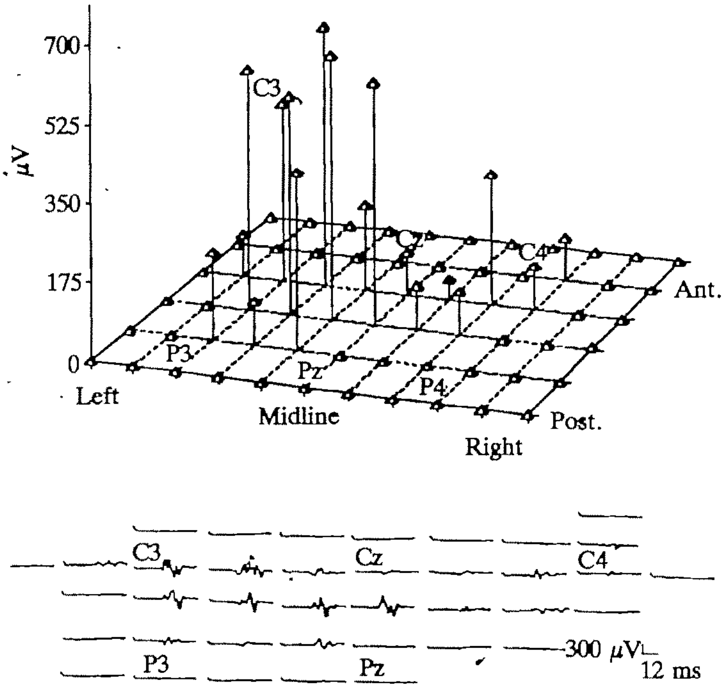

FIG 3. Left TM motor map in *Case 1* obtained by stimulating different scalp locations with the same stimulus duration and intensity (100 μs, 700 V) using the bipolar short-distance montage (*see* Methods for details). The diagram represents the top of the head. Triangles show the scalp positions where stimulating electrodes (anodes) were placed. The height of each triangle shows the mean amplitude (in μV) of 3 muscle responses recorded from the left TM with stimulation of each scalp position. EMG traces are examples of muscle responses recorded on stimulation of each scalp position. Note the similar latency of EMG responses evoked by stimulation of either side of the scalp and the presence of large EMG responses evoked by stimulation of the midline.

from the left TM were similar with stimulation of the right and left sides of the scalp (±1.8 ms) (fig. 3).

Motor representation areas for TA were determined only in Case 1. Maximal responses from the right and left TA were obtained when stimulating over Cz. These TA motor maps did not differ from those obtained in our normal volunteers (Cohen and Hallett, 1988*b*).

The maximal responses from the right TM of Case 2 were obtained when stimulating contralaterally 2.3 cm medial to C3 (1000 μV) and 2.3 cm behind C3 (850 μV). Stimulation over the midline (Cz, Pz) did not evoke any response from the right TM. Stimulation over C4 (420 μV) evoked moderate muscle responses from the right hand. This is a different scalp distribution from the one observed in Case 1 in that stimulation over the midline was ineffective in evoking responses from the right hand (fig. 4A).

The maximal responses from the left TM of Case 2 were obtained when stimulating ipsilaterally 2.3 cm medial to C3 (990 μV). Stimulation over the midline (Cz, Pz) did not evoke any response from the left TM. Stimulation over C4 evoked moderate muscle responses from the left hand (350 μV) (fig. 4B). As in Case 1, the latencies of EMG

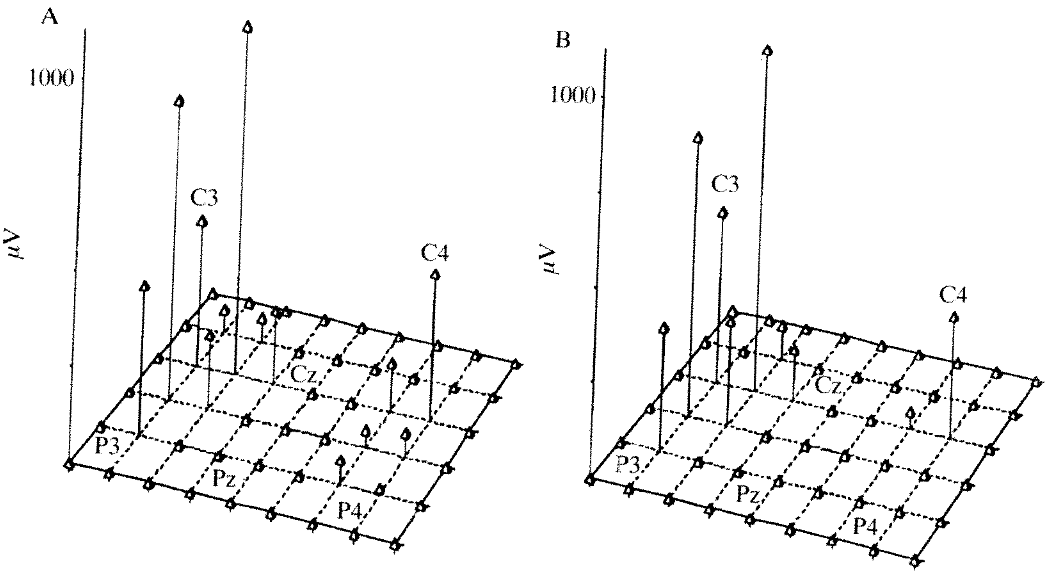

FIG. 4. Right hand (A) and left hand (B) motor maps recorded from *Case 2* and displayed as in figs 1 and 3. Note that the right and left TMs are activated by stimulation of either side of the scalp (100 μs, 600 V), but not the midline.

responses from the left TM were similar with stimulation of the right and left sides of the scalp.

Conduction time from brain to muscle

Electrical and magnetic stimulation in normal volunteers evoked contralateral muscle responses exclusively. In Case 1, the amplitude of maximal M responses from the right and left TM to electrical stimulation of the median nerve at the wrist was 5 mV bilaterally. Electrical stimulation delivered over C3 and C4 evoked muscle responses from both hands at similar latencies. The earliest latencies were 18.4 ms in both muscles after C3 stimulation and 17 ms in both muscles after C4 stimulation. Magnetic stimulation delivered over C3 and C4 also evoked bilateral muscle responses at similar latencies. The earliest latencies were 19 ms in both muscles after C3 stimulation and 18.5 ms after C4 stimulation.

In Case 2, the amplitude of maximal M responses recorded from the right and left TM to median nerve electrical stimulation was 9 and 10 mV on the right and left sides, respectively. Electrical stimulation delivered over C3 and C4 evoked muscle responses from both hands at similar latencies. The earliest latencies were 18.5 ms in both muscles after C3 stimulation and 18 ms in both muscles after C4 stimulation (fig. 5). Magnetic stimulation delivered over C3 and C4 also evoked bilateral muscle responses. The earliest latencies were 20 ms in both muscles after C3 stimulation and 19 ms in both muscles after C4 stimulation (fig. 6).

Premovement potentials

In normal subjects, the peak of NS′ occurred 95 ms before movement onset, the PMP occurred as an ipsilateral relative positivity after the peak of NS′, and the initial slope

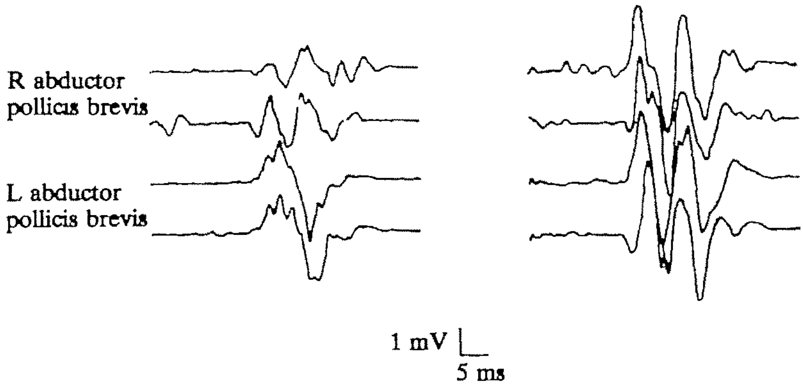

FIG. 5. EMG responses from right and left TMs obtained during mild muscle activation when stimulating electrically over C3 (*left*) and C4 (*right*) (100 μs, 600 V). Two traces are shown for each response, and the earliest recorded latencies were considered.

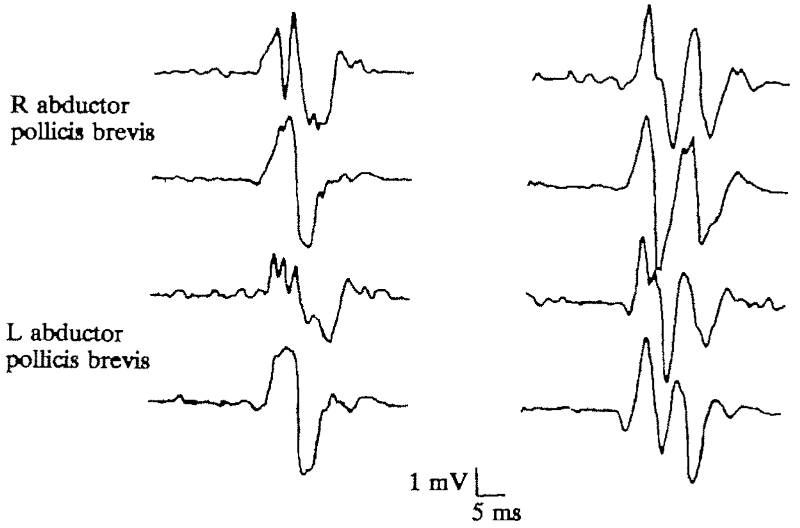

FIG 6. EMG responses from right and left TMs obtained during mild muscle activation when stimulating magnetically over C3 (*left*) and C4 (*right*) 90% of output of the magnetic stimulator, a Cadwell MES-10, 2.2 tesla. Stimuli delivered over C3 evoked muscle responses from right and left TM at similar latencies Stimuli delivered over C4 also evoked bilateral responses at similar latencies. Two traces are shown for each response, and the earliest recorded latencies were considered.

of MP occurred 4 ms before movement on the dominant side with a focal topography on contralateral precentral areas (Tarkka and Hallett, 1990).

The latencies of NS′, PMP, and the initial slope of MP recorded in the 2 patients were similar to those obtained in normal volunteers (fig. 7). However, major differences were observed in the topography. Both patients showed normal scalp distributions at the peak of NS′, but neither of them exhibited identifiable ipsilateral PMPs (fig. 8).

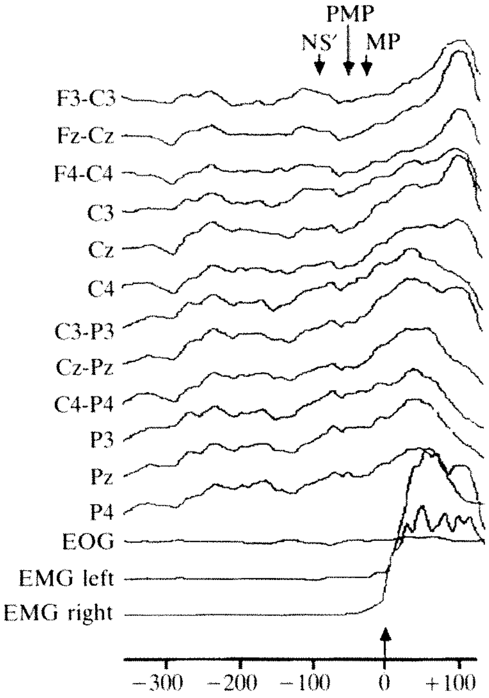

FIG. 7. Cortical potentials preceding voluntary abduction of right digit II in *Case 1*. Only 15 of 32 channels are shown for the sake of clarity. Recordings from channels labelled by 2 scalp positions (e.g., F3-C3) were obtained from the midpoint between both (*see* text for details). The movement onset occurred at 0, and the time scale is in ms. Arrows mark the potentials mapped in fig. 8. Note that both right and left index finger EMGs (rectified) show activity, although the task was unilateral.

The most striking finding was that the initial slope of MP started more bilaterally than in normal recordings, but still was prominent contralateral to the intended movement. In the next 10 ms, the initial slope of MP shifted to an abnormal bilateral scalp distribution in both patients (fig. 9).

Somatosensory evoked potentials

We identified different components of the SEPs elicited by electrical stimulation of the right and left median nerves and labelled them according to their latencies (fig. 10, Table 1). Several components were identified: P12, contralateral postcentral N17, contralateral central P19, contralateral P21, anteromedial N25 and P35 (figs 10, 11). In Case 1, the P40 component was maximal over Cz with right and left tibial nerve stimulation, with a predominantly ipsilateral scalp distribution. Careful analysis has failed to reveal any differences in scalp distribution of patients and controls.

Voluntary movements and stretch reflexes

None of the 5 normal volunteers exhibited EMG activity in the muscles of the hand contralateral to the one performing the voluntary movement. When the patients performed the voluntary movement with the right hand, a triphasic EMG pattern was seen in the

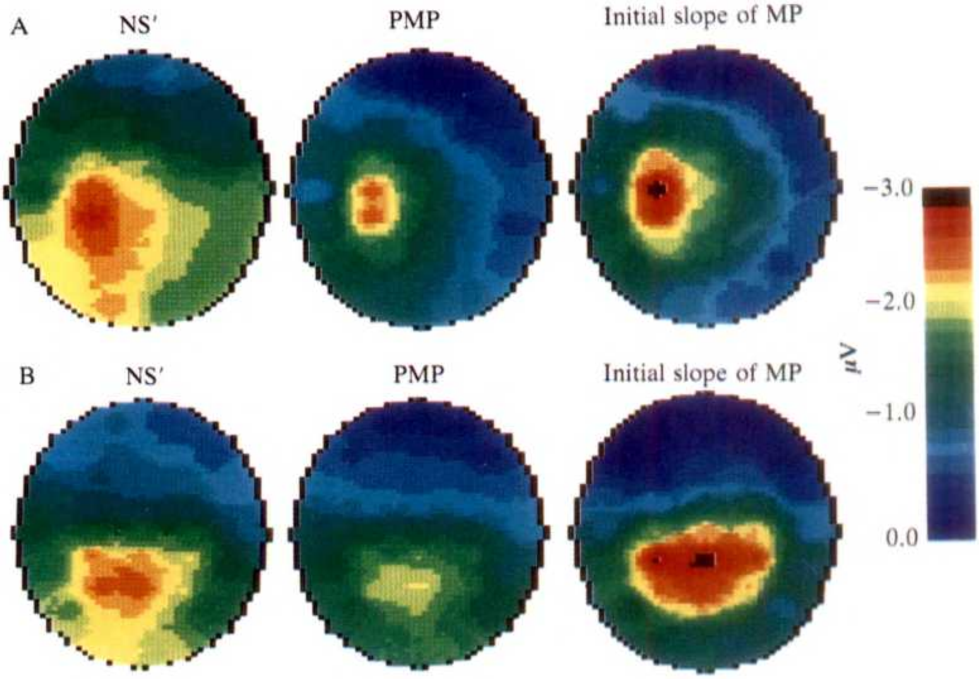

FIG. 8. Topographic maps of the peak of NS′, PMP, and the initial slope of MP preceding voluntary abduction of the right digit II in a normal volunteer (A) and *Case 1* (B). PMP is identified by positivity in the ipsilateral cortex region and this is not seen in the patient. Note the bilateral scalp distribution of the initial slope of MP in the patient in comparison with the normal volunteer.

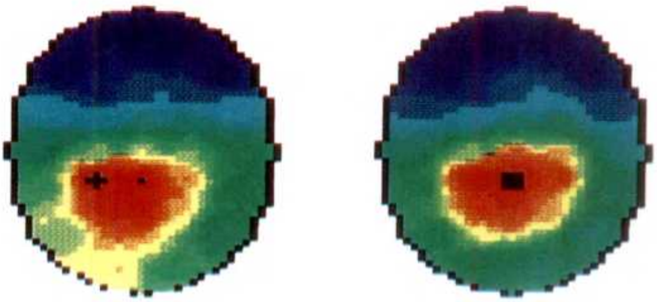

FIG. 9. Scalp distribution of the initial slope of MP with intended abduction of the right digit II by *Case 1*. The map on the *left* shows the distribution of the initial slope of MP more prominent in the contralateral central area. The map on the *right* shows the distribution 10 ms afterwards. Note the abnormal spread of activity in both hemispheres.

flexor and extensor muscles of the active and 'mirror' hands at an equal lead time of 65 ms (Case 1) and 66 ms (Case 2), before movement onset (fig. 12).

There was an obvious distinction between first and second agonist bursts of the mirror hand in Case 1, but not in Case 2. When voluntary movements were made with the left nondominant hand, a triphasic EMG burst was also seen in the active and mirror

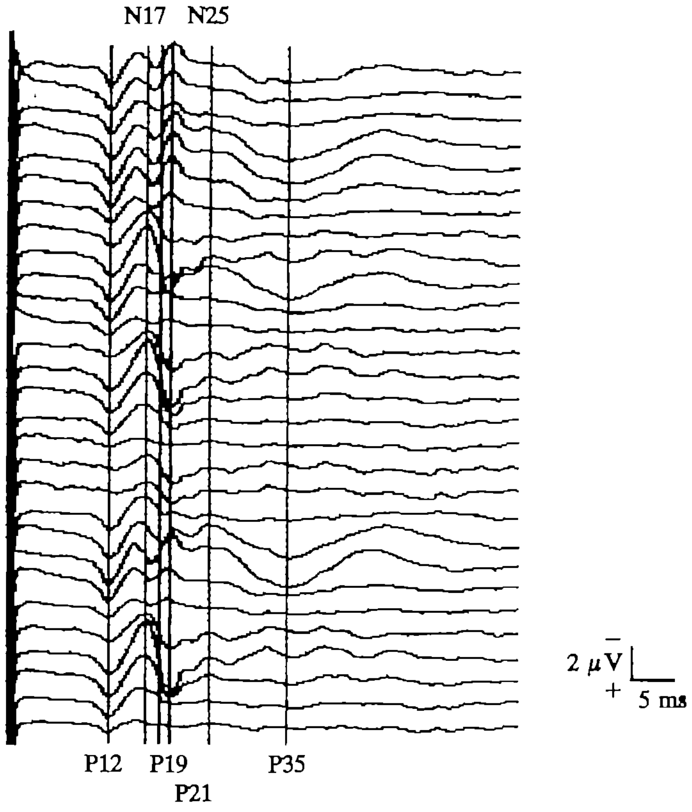

FIG 10. SEPs elicited by stimulation of the right median nerve in *Case 2*. Average of 2000 stimuli. Bars indicate the components mapped in fig. 11. Recordings were obtained from 29 scalp positions referenced to linked earlobe electrodes: Fp1, Fp2, F7, F3, Fz, F4, F8, T3, C3, Cz, C4, T4, T5, P3, Pz, P4, T6, O1, O2 and midpoints between F7-T3, F3-C3, Fz-Cz, F4-C4, F8-T4, T3-T5, C3-P3, Cz-Pz, C4-P4, T4-T6

TABLE 1. LATENCY OF SOMATOSENSORY EVOKED POTENTIAL (ms)

Component	*P12*	*N17*	*P19*	*P21*	*N25*	*P35*
Case 1 R	11.50	17.50	*	25.50	27.50	35 75
L	11 50	17.25	20.00	26.75	*	33 50
Case 2 R	12.75	17.25	19.00	20.50	25.50	35.50
L	12 75	17.00	18.50	20 25	27.75	35.25

* Not identified.

wrist muscles. In contrast to movements actively performed with the dominant hand, the onset of muscle activity with movement of the mirror hand was delayed by a mean of 14 ms in Case 1 and 19 ms in Case 2 in relation to EMG onset from the active hand. The mirror hand of both patients did not show a distinctive second agonist burst when the active hand was moving.

When the patients performed extension of the right digit III, the mirror activity occurred

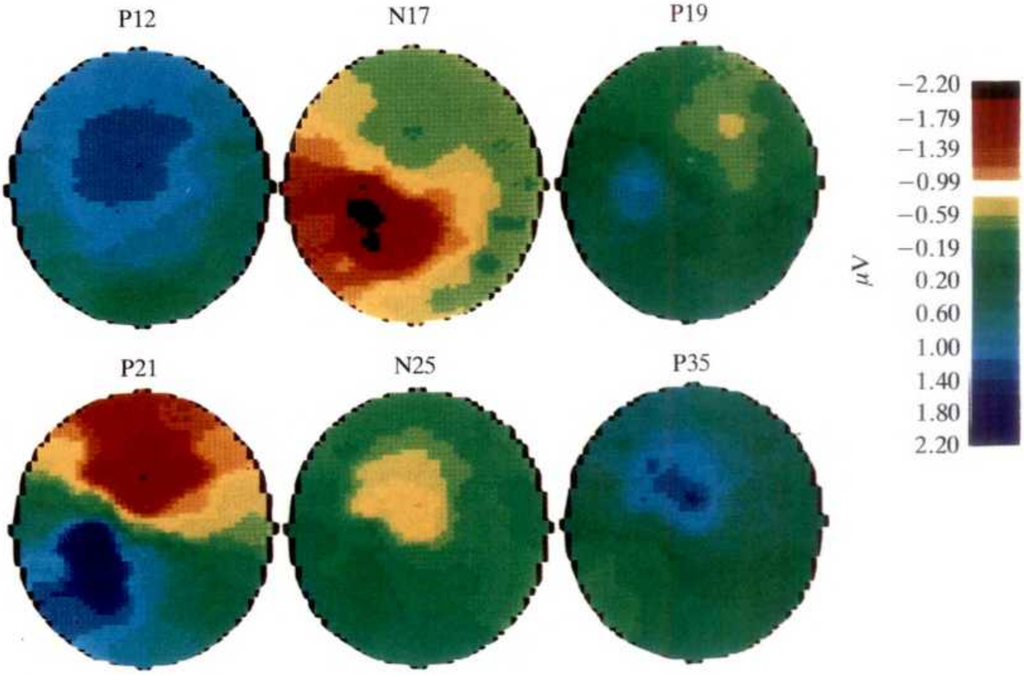

FIG. 11. Scalp distribution of SEPs elicited by stimulation of the right median nerve in *Case 2* (same as in fig. 10). Each colour represents a certain amplitude range, as shown on the left side of the colour scale at the right. Each map was constructed at the latencies shown by the bars in fig. 10. Note the precentral distribution of P12, the clearly postcentral and contralateral N17, the well-localized contralateral and central P19, and the clearly contralateral P21.

at a mean of 6 ms (Case 1) and 29 ms (Case 2) after the onset of the active movement. When the patients performed extension of the left digit III, the mirror activity occurred at a mean of 8 ms (Case 1) and 42 ms (Case 2) after the onset of the active movement. With the 3 paradigms studied, EMG activity from the mirror movement in individual trials started as fast as that from the intended movement.

When the right hand was extended involuntarily, short-latency responses from flexor muscles were evoked in that hand in both patients (19 and 25 ms in Cases 1 and 2, respectively), but long-latency responses occurred only in Case 2 (62.5 ms). The same stimulus evoked occasional small, short-latency responses in the mirror hand at a latency of 26 ms in Case 1. When the left hand was extended involuntarily, short-latency muscle responses from flexor muscles were evoked in that hand in both patients (9 ms in both), but long-latency responses occurred only in Case 2 (50 ms). No EMG responses were seen in the mirror hand in either patient. None of the 5 normal volunteers showed mirroring of the stretch reflex to the other hand.

Positron emission tomography

Tissue activity estimated with the method we used accurately reflects blood flow in physiological ranges, although underestimation may occur at higher flow values (Mazziotta *et al.*, 1985). With movement, the normal subjects showed increments in

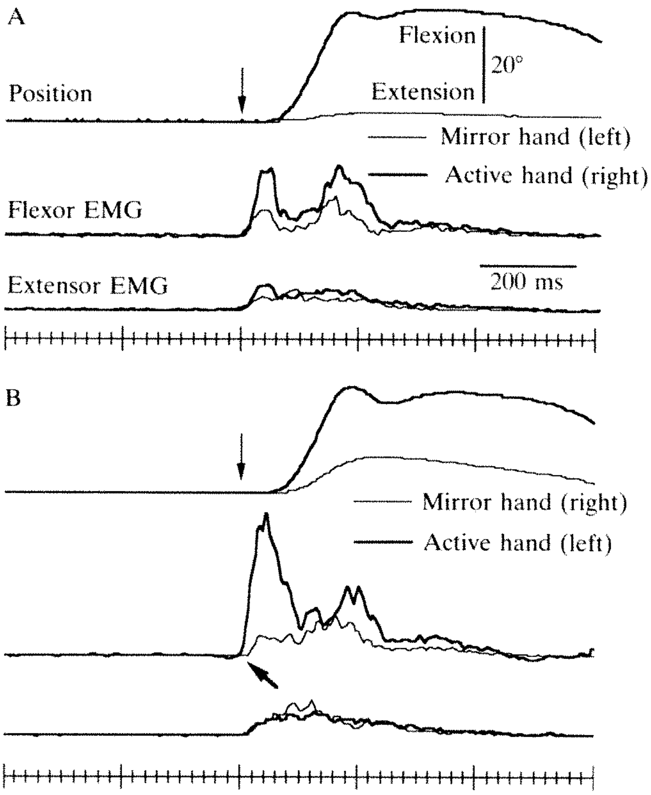

FIG. 12. Averaged hand position and rectified filtered EMG responses in flexor and extensor muscles during voluntary wrist flexion of the right, dominant, hand (A) and of the left, nondominant, hand (B). Movement and EMG recordings from the inactive or 'mirror' hand (thin lines) are superimposed on recordings from the active hand (thick lines). The vertical arrows point to the onset of the agonist EMG response, and the slanted arrow shows the lag in onset latency of the EMG related to the mirror hand with respect to the active hand.

contralateral motor cortex blood flow of 0–29%, with an average of 16% (Table 2). The meaning of a failure to detect an increase is uncertain, since the hand region of the motor cortex could have been missed or only partly sampled by the limited number of slices in each scan. For example, in the subject with 0% increment, it is certain that the top slice of the PET scan was too low to include the hand region. Also, there was an intraindividual variance of 10% in the 2 measurements of the normalized motor cortex ROI in the rest condition. Given this, it does not seem reasonable to accept less than a 10% increase as indicative of identifiable motor cortex activation. For this reason, we calculated a ratio of the increment in ipsilateral to contralateral motor cortex blood flow for only those subjects with increments in the contralateral motor cortex of at least 10%. Two of the 11 normal subjects were therefore excluded. In the remaining 9 subjects, the ratio varied from −0.27 to 0.24, with a mean of 0.11. (Complete results from the normal subjects will be published elsewhere.) Case 2 was also excluded by our criterion, given an increment in contralateral cortex blood flow of only 9%. Case 1 showed marked activation of ipsilateral as well as contralateral motor cortex when attempting unilateral

TABLE 2. MEASUREMENTS OF CHANGES IN BLOOD FLOW IN MOTOR CORTEX ON MOVEMENT

Subject	*% activation of contralateral cortex*	*Ratio of ipsi vs contralateral activation*
NV1	29	0.21
NV2	27	−0.21
NV3	20	0.13
NV4	10	−0.29
NV5	20	−0.03
NV6	15	0.01
NV7	17	0.25
NV8	19	0.11
NV9	18	0.02
NV10	5	0.31
NV11	0 (not identified)	
Case 1	15	1.09
Case 2	9	−0.26

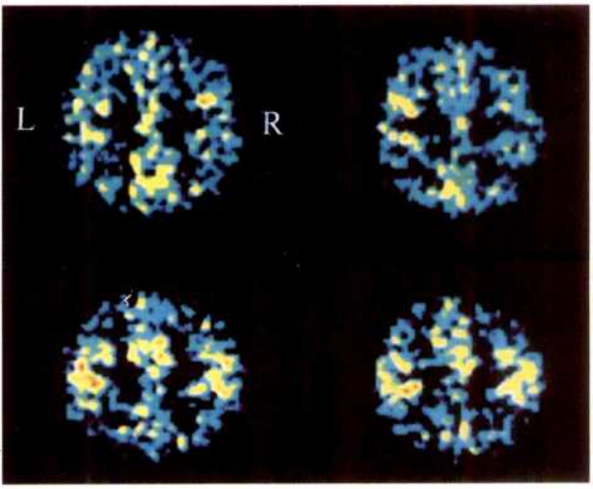

FIG. 13. PET scan obtained from *Case 1* during rest (*top*) and right wrist movement (*bottom*). Note the bilateral activation of central areas together with midline during intended unilateral right wrist movement.

movement (and exhibiting bilateral movement) (fig. 13). The ratio for Case 1 was 1.09, which was markedly higher than for any control.

DISCUSSION

Because the term mirror movements has been used to describe the physiological mirror movements seen in infants, the relatively pure congenital movements, and those associated with other neurological abnormalities, it is particularly important to characterize the patients we studied. They belong to the group of patients who have congenital mirror movements (a separate entity from 'associated or bimanual' movements). Case 2, for example, could only master the crawl swimming technique after years of practice, but was able to use the breast stroke after a few weeks of practice. The breast stroke requires that both arms perform a clear mirror movement, while the crawl requires differentiated, independent control of both upper extremities. As in patients previously reported (Schott and Wyke, 1981), both our patients had trouble typing and 1 (Case 2) had difficulty playing the piano. Mirror movements were seen when both patients intended unilateral finger or, to a lesser extent, wrist movements. However, proximal arm movements were not usually mirrored. With unilateral finger movements, the extent of the mirroring was similar to the intended one. Mirror movements in our patients were not present with unilateral passive finger displacement, as reported in other patients (Drinkwater, 1914; Guttmann *et al.*, 1939; Schott and Wyke, 1981). No other neurological disorder was associated with the mirror movements, but Case 2 has had severe depression. The mirror movements were identified in the patients' early infancies, and there was no significant progression. In Case 2, genetic transmission was evident and was autosomal dominant with incomplete penetrance. Case 1 seems to represent a sporadic case. MRI in both patients excluded the presence of agenesis of the corpus callosum. Both patients learned to suppress some of the mirror activity.

The results from the different tests performed in these 2 patients show that (1) muscle responses in both hands can be evoked by stimulation of each cerebral hemisphere, (2) ipsilateral and contralateral MEPs elicited by unilateral scalp stimulation have similar latencies, (3) the initial slope of MP, a premovement component, presented with an abnormally bilateral scalp distribution, (4) blood flow increased in both ipsilateral and contralateral motor cortices with attempted unilateral movement, (5) intended movement of either hand evoked EMG activity in the other hand as early as simultaneously, and (6) scalp distribution of SEPs elicited by median nerve stimulation was normal in both patients.

We found that unilateral scalp stimulation was unable to evoke ipsilateral muscle responses from the hand in normal volunteers (Cohen and Hallett, 1988*a*), which also was true for direct cortical stimulation during surgical exploration of the brain (Penfield and Jasper, 1954; Woolsey *et al.*, 1979). The finding that muscles of each hand can be activated by stimulation of ipsilateral or contralateral motor cortex in these 2 patients suggests the existence of a bilateral cortical representation for hand muscles. Moreover, in Case 1, stimulation of the midline also evoked large EMG responses from the hand. This finding suggests that, at least in this patient, the classic homuncular organization of the motor cortex is deranged, with enlargement of the hand motor representation area over the regions of the shoulder, trunk, and perhaps leg.

These results indicate abnormal connectivity between the motor cortex and specific muscle groups. This deranged 'map of outputs', in a sense similar to that given to this concept by Phillips (1987), indicates the presence of physiologically active connections between the motor cortex and distal hand muscles in the ipsilateral upper extremity. Moreover, these connections are able to conduct efferent volleys at fast conduction velocities, as fast as those travelling along the crossed corticospinal tract in normal subjects. It is possible that efferent volleys in patients with mirror movements travel along physiologically active ipsilateral pathways. In humans, approximately 1 million corticospinal fibres pass through each medullary pyramid, with 70–90% crossing in the motor decussation and originating the lateral corticospinal tract. This is particularly important for the performance of distal fine contralateral finger movements, precisely the type of movements more affected by mirror movements (Lawrence and Kuypers, 1968). However, approximately 10–30% of the fibres remain uncrossed, originating the ventral corticospinal tract (Nathan and Smith, 1973). If the ipsilateral MEP is actually conducted through ipsilateral pathways, it is possible that the percentage of uncrossed fibres is larger than normal. This possibility is favoured by the association of mirror movements in a patient with the Klippel-Feil syndrome with the absence of pyramidal tract decussation at autopsy (Gunderson and Solitare, 1968) and by the persistence of congenital mirror movements in a patient after a stroke left an absolute flaccid paralysis of the limb (Haerer and Currier, 1966). Perhaps these usually inactive or not evidently active ipsilateral projections become active under some pathological conditions, such as those decribed by Jung and Dietz (1975). They found delayed onset of intended movements on the affected side of patients with unilateral pyramidal tract lesions. However, if the patients intended a simultaneous bilateral movement, the latency delay disappeared. They attributed this finding to the activation of ipsilateral corticomotoneuron projections.

It is unlikely that interhemispheric connections are responsible for the simultaneous ipsilateral response to cortical stimulation. Two different lines of evidence suggest that conduction through the corpus callosum of humans requires at least 10 ms. First, Amassian and Cracco (1987) recorded a 'transcallosal response' at this latency when electrically stimulating the contralateral scalp. Secondly, Shibasaki *et al.* (1978) and Wilkins *et al.* (1984) showed that EMG bursts separated by 10 ms in different arms of patients with myoclonus were correlated with cortical activity 10 ms apart in the hemisphere contralateral to myoclonic movements and attributed this delay to transcallosal transmission. Our studies showed, however, that unilateral scalp stimulation evoked bilateral muscle responses at similar latencies, clearly not allowing enough time for conduction through transcallosal connections.

The analysis of premovement potentials in patients with mirror movements indicates that unilaterally intended voluntary movement results in an abnormal bilaterally distributed initial slope of MP but no clear abnormality of earlier components. The initial slope of MP is normally focused over the contralateral precentral hand representation area and may represent the activation of primary motor cortex (Neshige *et al.*, 1988; Tarkka and Hallett, 1990). Its bilateral distribution in our 2 patients is similar to that shown with mapping of MEPs with transcranial stimulation. Shibasaki and Nagae (1984) studied premovement potentials in a patient with mirror movements. They recorded the entire Bereitschaftspotential with less interest in the deflections close to movement onset. The early preparation to move appeared normal in their patient, and a clear PMP was impossible to find. They reported that the peak of NS′ occurred bilaterally with contralateral preponderance. We saw the bilaterality in the initial slope of MP, but used a different method of analysis. Two mechanisms could be involved in the bilaterality in the initial slope of MP: (1) for voluntary movement of a limb, both primary motor cortices are activated simultaneously, or (2) one primary motor cortex is activated first and then the motor command spreads to the other side. The first possibility is conceivable since, as discussed before, conduction times from cortex to ipsilateral and contralateral muscles were equal, not allowing time for interhemispheric conduction. However, the progression from contralateral to bilateral in the genesis of the initial slope of MP suggests that the motor command starts in the contralateral motor cortex and rapidly spreads to the ipsilateral motor cortex. It is likely that patients with congenital mirror movements exhibit anatomical abnormalities in motor tracts and develop different strategies for motor control than do normal subjects involving bilateral cortical activation when unilateral movements are intended.

Evidence for mirror movements originating in abnormal interhemispheric pathways is lacking (Schott and Wyke, 1981). However, the association of agenesis of the corpus callosum and mirror movements has been known for a long time (Freiman *et al.*, 1949; Ettlinger *et al.*, 1972), and it has provided support for the theory of transcallosal inhibitory pathways being responsible for inhibition of ipsilateral movements in normal adults. Patients with callosal abnormalities would lack this inhibitory pathway and consequently perform ipsilateral movements on intended contralateral motor commands. Agenesis of the corpus callosum is also associated with pyramidal tract abnormalities (Parrish *et al.*, 1979), and perhaps it is these abnormalities that provide the basis for the occurrence of mirror movements (Schott and Wyke, 1981).

Activation of both ipsilateral and contralateral motor cortex with voluntary movement was suggested by both premovement and PET studies. We cannot be certain about the precise anatomical regions involved with PET given the resolution of the method and the lack of a correlative imaging study, such as an MRI scan. Nevertheless the evidence for motor cortex activation during movement is overwhelming (Arezzo and Vaughan, 1980; Lee *et al.*, 1986; Neshige *et al.*, 1988). A similar conclusion has been drawn by Roland (1984). Possible contributions by the premotor cortex or primary somatosensory cortex, however, cannot be disregarded. Placement of the ROI on the ipsilateral cortex was at a symmetric contralateral position, so that changes there should reflect similar anatomical sites. Weak activation of the ipsilateral cortex appears to occur in some normal subjects but, with our procedure, only up to about 24% of the contralateral side is involved. Activation of the ipsilateral cortex in Case 1 was even greater than that of the contralateral cortex.

Mirror activity occasionally occurs with extreme effort in normal subjects (Cernacek, 1961; Kristeva *et al.*, 1979; Schott and Wyke, 1981). However, none of our controls showed any clinical or subclinical mirroring when performing mild wrist flexion and mild finger extension or abduction, tasks that are far from requiring extreme effort. EMG findings in patients with mirror movements have been described (Cernacek, 1961; Green, 1967; Regli *et al.*, 1967; Jelasic and Ott, 1969; Chaco and Blank, 1974; Hopf *et al.*, 1974; Forget *et al.*, 1986), and the time relation of the onset latencies of intended and mirror EMG bursts is a matter of debate. Both Regli *et al.* (1967) and Jelasic and Ott (1969) found that on average the EMG of the mirror movement was slightly delayed (15 and 35 ms in 2 patients with Regli *et al.*). Forget *et al.* (1986), however, reported identical onset latencies in EMG bursts for intended and mirror movements of 2 patients. In our study, the onset latency of mirror EMG activity occurred as early as simultaneously with that of the intended activity. This finding is compatible with transmission of the motor command through fast ipsilateral corticomotoneuron connections or with fast interhemispheric transmission (with an ipsilateral-contralateral delay of less than 10 ms).

We found no mirroring in the long-latency muscle reflexes. If we assume that the afferent limb of the stretch reflex relays its neural messages to cortical output cells (Evarts, 1981), we might expect that the long-latency stretch reflex would be 'mirrored'. The fact that it is not could mean that this type of stimulation is unable to activate connections between the motor cortex and ipsilateral muscles in the arm or that it is not mediated through a transcortical pathway. The absence of mirroring in the myotatic reflex in these patients suggests that in relation to spinal cord circuitry, at least, Ia afferents are limited in distribution to the ipsilateral side.

We have found that the abnormally large representation of the hand in the motor cortex is not correlated with similar findings in the sensory cortex. The scalp distribution of SEPs to electrical stimulation of median and tibial nerves were no different in our patients than in controls. The median nerve SEP components P12, N17, P19, P21, N25 and P35 are similar to P14, N20, P22, P27, N30 and P45 (Desmedt and Bourguet, 1985). The reason for our earlier latencies probably lies in the more proximal site of stimulation in our study (median nerve compared with digital nerve) and in the fact that our 2 patients had relatively short stature. The scalp distribution of the postcentral N17 and P21 were clearly contralateral, as well as the central P19, N25 and P35 component. N17 (N20) is recognized as the scalp reflection of the initial cortical response to the afferent sensory volley (Allison *et al.*, 1980). The fact that it showed a contralateral scalp distribution in these two patients suggests lack of bilateral projection of afferents. It has been proposed that the P19 (P22) component could represent the projection of fast conduction afferent fibres from the thalamus to area 4 (Desmedt and Cheron, 1981; Mauguière *et al.*, 1983). If this is the case, these sensory projections seem also to be clearly contralateral. It is possible that these conclusions apply only for cutaneous afferents, while muscle afferent projections may or may not be bilateral.

In summary, the cortical stimulation studies showed that there are physiologically active ipsilateral, as well as contralateral, motor pathways, indicating an abnormality in the anatomical circuitry. Moreover, the study of premovement potentials and the PET studies showed that both ipsilateral and contralateral motor cortices are activated with voluntary movement. Support for involvement of the ipsilateral pathway in congenital mirror movements comes from several lines of evidence. First, the study of premovement potentials revealed initially predominant activation of the motor cortex contralateral to the willed movement with only subsequent spread to the ipsilateral motor cortex over the next 10 ms. Secondly, the EMG studies showed that the mirror activity started as early as simultaneously with the willed movement, suggesting that the ipsilateral motor pathway must be active in this task, since there is an obligatory delay of 10 ms for activation of the contralateral pathway. The third piece of evidence comes from a patient with stroke whose paralysed arm moved only as a mirror to contralateral willed movement (Haerer and Currier, 1966). After the movement begins, the motor cortex ipsilateral to the intended movement is activated and should be able to support the mirror activity. Activation of both motor cortices represents an abnormality distinct from activation of both ipsilateral and contralateral pathways from each hemisphere. Maps of SEPs were normal, as were long-latency stretch reflexes, suggesting that motor cortex activation via sensory cortex activation, if this is the pathway or contributing pathway to long-latency stretch reflexes, was also normal.

ACKNOWLEDGEMENTS

We thank the patients for their kind tolerance during these studies. Dr John Schwankhaus and Dr Bill Theodore offered helpful comments, and Ms B. J. Hessie provided editorial assistance.

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(*Received August 1, 1989. Revised January 30, 1990. Accepted March 6, 1990*)

NOTE ADDED IN PROOF

Mirroring of long-latency reflexes of finger muscles in 3 patients with congenital mirror movements was found by Capaday *et al.* (1989). This may indicate a difference between distal and more proximal muscles in this regard. The result is consistent with the notion that long latency stretch reflexes of the fingers are more likely to be transcortical than the reflexes of more proximal muscles Thus for finger muscles, sensory cortical afferents may well activate bilateral motor pathways.

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PERCEPTION AND ACTION IN 'VISUAL FORM AGNOSIA'

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SUMMARY

A single case study of a patient with 'visual form agnosia' is presented. A severe visual recognition deficit was accompanied by impairments in discriminating shape, reflectance, and orientation, although visual acuity and colour vision, along with tactile recognition and intelligence, were largely preserved. Neuropsychological and behavioural investigations have indicated that the patient is able to utilize visual pattern information surprisingly well for the control of hand movements during reaching, and can even read many whole words, despite being unable to make simple discriminative judgements of shape or orientation. She seems to have no awareness of shape primitives through Gestalt grouping by similarity, continuity or symmetry. It is proposed that many of these perceptual disorders might be the combined result of (1) a selective loss of the cortical elaboration of the magnocellular visual processing stream, and (2) a selective output disconnection from a central processor of visual boundaries and shape primitives in the occipital cortex.

INTRODUCTION

Benson and Greenberg (1969) described a syndrome which they named 'visual form agnosia'. Their patient (Mr S.) had severe object-recognition difficulties, which apparently could not be explained by serious sensory or intellectual impairment. He had poor copying ability, which would tend to put him into the category of apperceptive rather than associative agnosia (Lissauer, 1890). However, Efron (1969) showed that Mr S. had a more basic perceptual disability than would be expected within classical ideas of agnosia, in that he was unable to distinguish reliably between simple geometric shapes. This shape-perception deficit is implied in the introduction of the term 'form agnosia' by Benson and Greenberg, although Efron himself questioned the appropriateness of using the term 'agnosia'.

Other patients have been described in the literature with similar symptom pictures, the fullest accounts being of H.C. (Adler, 1944, 1950) and R.C. (Abadi *et al.*, 1981; Campion and Latto, 1985). Like Mr S., these 2 cases had suffered from anoxia and carbon monoxide poisoning (*see* review by Campion, 1987). More recently, Warrington (1985*a*, *b*) has reported 2 further patients (J.A.F., R.B.C.) who performed poorly on Efron's shape discrimination task, in spite of intact visual acuity and point localization. They were tested after a period of recovery from cortical blindness attributable to bilateral

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occipital infarctions, and would seem to fall into the same category as Mr S. Warrington (1985*b*) classified them as 'pseudoagnosic', along with other patients whose impaired object recognition could be attributed to primary sensory disorders.

Irrespective of terminology, it is generally agreed that these patients should be distinguished from those who have a visual recognition difficulty in the *absence* of impaired shape discrimination (e.g., the patients described by Warrington and James, 1988). This distinct and profound disorder of shape perception merits a name of its own to distinguish it from other causes of impaired object recognition. Both 'visual form agnosia' (Benson and Greenberg, 1969) and 'shape agnosia' (Humphreys and Riddoch, 1987*a*) indicate its specificity, whilst 'pseudoagnosia' does not; however there is force in Warrington's argument against the use of the word 'agnosia' in this context. It may be that a neutral term like 'dysmorphopsia' (impaired vision for shape) would ultimately be a suitable solution to the problem.

Following Efron's (1969) ideas, Warrington (1985*a*) and Humphreys and Riddoch (1987*b*) have argued that patients of this type have a disorder at a stage of perceptual processing where an initial 2-dimensional description of visual shapes is being constructed. Campion and Latto (1985) suggested more radically that a patchy ('peppered') array of small scotomata across the visual field could account for the disorder. We describe here a new case of visual form agnosia, who has recently undergone an intensive series of visual tests. These studies have uncovered a visual disorder of greater complexity than has been previously described.

CASE REPORT

D.F. was born in Scotland (in 1954) and received education in the UK and abroad up to college level, gaining a BA degree in business management. She had a private pilot's licence; and had worked in administrative posts and recently in freelance commercial translation. At the time of her accident, D.F. was aged 34 yrs and was living in northern Italy. She collapsed and lost consciousness while taking a shower at her home, due to carbon monoxide intoxication from a leak in a faulty propane gas water heater. She was admitted to hospital in deep coma with dilated pupils, and received urgent treatment with oxygen, dexamethasone and dextran infusion. She regained consciousness and was transferred after 2 days to the neurological clinic of the Ospedale Maggiore in Novara. On examination there, D.F. appeared blind, with loss of the eyelid response to menace; however, the pupillary light reflex persisted, and the fundi were normal. The cortical blindness began to fade after about 10 days, when it became clear that bright colours could be named; but D.F. continued to show severe difficulties with ocular fixation and pursuit. It was also apparent that she had poor memory, acalculia and disorientation in space and time. She had relatively preserved verbal skills and general knowledge, and no indication of neglect or sensory extinction. After 5 wks in hospital in Novara, D.F. returned to Scotland, where she stayed for 8 mos before returning once more to Italy.

CLINICAL INVESTIGATIONS

CT scans

At 11 days. Small low-density areas were seen on the right at the level of the internal capsule and around the body of the left lateral ventricle. No enhancement appeared following intravenous contrast.

At 9 wks. No clear abnormality was observed.

SPECT

At 4 wks. There was evidence of reduced cortical blood flow in the left posterior parietal and temporo-occipital regions, especially at the temporo-occipital junction (*see* fig. 1). There was also evidence of widespread hypoperfusion in the frontal area on both sides.

At 8 mos. Symmetrical hypoperfusion was then reported bilaterally in parieto-occipital regions, with no obvious abnormality in the striate cortex, or in the frontal or temporal lobes.

MRI

The spin-echo technique was used on both occasions of testing.

At 17 days. The lateral ventricles were of normal shape and dimensions, and the third and fourth ventricles lay in the midline, but there was evidence of damage at the level of the lentiform nucleus bilaterally and, less clearly, in temporo-occipital cortex on the left side.

At 13 mos. An altered signal was seen in the globus pallidus bilaterally and, very clearly, in the occipital region bilaterally. This was mainly on the lower aspects of the lateral occipital cortex and at the polar

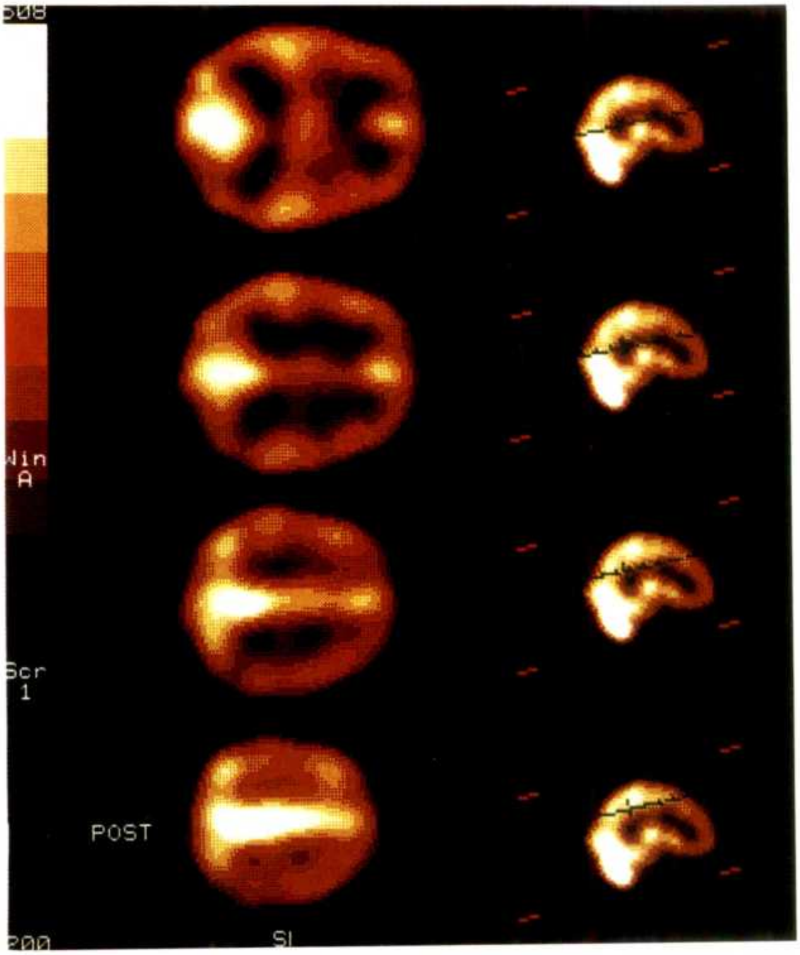


FIG. 1. SPECT images recorded at an early stage (1 mo). Four horizontal sections through the cortical lesion, presented with posterior to the left and with left/right reversed.

convexity (*see* fig. 2), extending into the parasagittal occipitoparietal region. Also a widening of the ventricles and sulci is apparent.

EEG

At 3 wks. Increased bilateral slow wave activity was apparent posteriorly.

At 10 wks. There was a low-amplitude EEG with a poor alpha rhythm, an excess of theta components in the temporal and occipital regions, and occasional small slow waves in both temporal regions, maximal and phase-reversing at the right midtemporal electrode. These features are consistent with diffuse anoxic damage.

Summary

In the early stages of the disease, neither CT nor MRI scans revealed the posterior cortical lesion which became clearly apparent in the later MRI (*see* fig. 2). However, there was a clear indication of this cortical pathology in both the early and the later SPECT scans.

NEUROPSYCHOLOGICAL TESTING

At Novara

Several tests were administered both during D.F.'s initial stay in hospital in Italy (at about 1 mo. postaccident) and then again approximately 12 mos after the accident.

Eisenson Battery. No evidence was found of aphasia (sentence and story comprehension) on either occasion, but clear evidence of a deficit on the agnosia subtests, with zero scores on visual recognition at 1 mo., despite comparatively good auditory (6/6) and tactile (4/6 left, 4/6 right hand) recognition. At 12 mos, hearing and touch were at 100% correct, but visual recognition remained impaired (common objects 4/6, drawings 0/4; shapes 1/4; small-size drawings 0/4; but colours 5/5).

Gelb/Goldstein tests. The coloured skeins were correctly pointed to only in the lower visual field, at 1 mo.; they were all indicated correctly at 12 mos. When presented with 20 objects together, D.F. was able to identify none without prompting at 1 mo.; at 12 mos she correctly identified 7/20. Objects indicated by the examiner were identified verbally at a level of only 4/10. In contrast, objects named by the examiner were pointed to accurately (17/17), though with hesitation. (A similar dissociation was noted informally between naming indicated letters and pointing to named letters Presumably in the pointing task a search for the 'target' could proceed by comparing the stimuli on a simple feature or features, whereas correct performance in the naming version would require a more complete perceptual analysis.) On the Gelb/Goldstein verbal classification tests, performance was predominantly good.

Wechsler-Bellevue scale (Italian: form 1). D.F.'s verbal IQ was average at 1 mo. on most subtests (Information, Comprehension, Analogies and Vocabulary), but low on Arithmetic (scaled score of 2) and Digit Span (1). At 12 mos, scores on 2 subtests had risen to a high level (Comprehension 16 and Analogies 14) but Arithmetic (3) and Digit Span (4) remained low.

Other cognitive tests. Historical and geographical knowledge was patchy, though the latter had improved greatly at 12 mos. Severe acalculia was apparent at 1 mo. on the Milan test, though this showed substantial improvement at 12 mos.

Memory was tested by various means, and was found to be low for spatial span (Corsi block-tapping task, tested at 12 mos only: span of 2), Wechsler Memory Scale difficult paired associates (score of 0 on both test occasions), verbal free recall (no recall at 1 mo., impaired primacy at 12 mos), and story recall (0 score at 1 mo.).

In De Renzi's modification of the Weigl sorting test (administered at 12 mos only) D.F. could not produce any self-initiated classification of the blocks. When the examiner sorted them in different ways, D F. correctly identified some sorting criteria visually (colour, and size after long reflection) but not others (shape, thickness or suit).

At St Andrews

The tests began 3 mos after the accident

Object recognition. A severe disorder of object recognition was confirmed, with several errors being made with everyday items (e.g., a cup misidentified as an ashtray, a fork as a knife). Some objects could

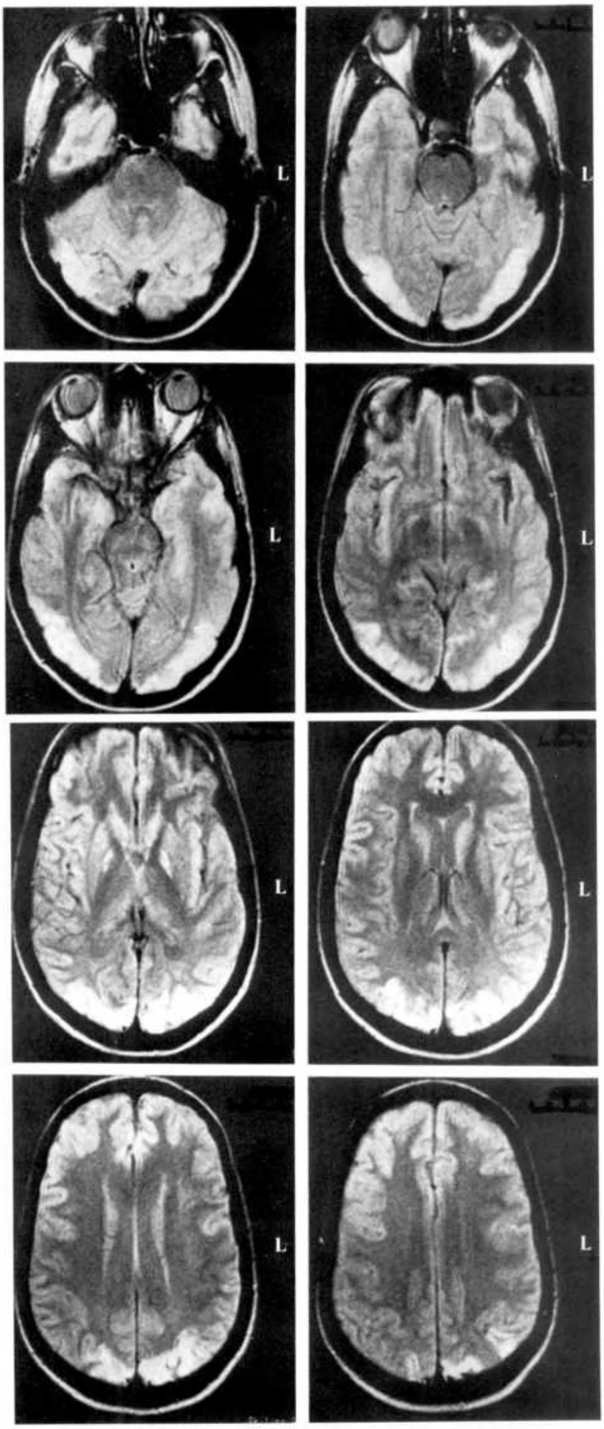


FIG. 2. Magnetic resonance images recorded at 13 mos. Horizontal sections through the cortical lesion, spaced 9 mm apart, are shown (left/right reversed).

not be named but could be partially described (e.g., a screwdriver as 'long, black, thin', and a pair of scissors as 'long, thin, silver'). D F. was able to make intelligent guesses on the basis of such features, but this tended to be a slow process In all cases the objects were readily identified tactually.

Recognition of drawings. Standard drawings of common objects (Snodgrass and Vanderwart, 1980) projected onto a screen could not be identified at all. Attempts to copy drawings of a simple house and of a Necker cube produced impoverished copies consisting of a few unconnected lines. The difficulty in recognizing line drawings was apparent at 4 mos even in a multiple-choice task (the Peabody Picture Vocabulary Test, which requires pointing to 1 of 4 pictures in response to a word). Performance was equivalent to a mental age of only 2 yrs 2 mos. By 8.5 mos postaccident, a comparable test (the short form of the British Picture Vocabulary Scale) gave an improvement up to a mental age score of 6 yrs 5 mos, with much hesitation and 'educated guessing'. Even this improved score is far below the level expected from the above-average WAIS vocabulary scaled score of 11

Intelligence. WAIS verbal IQ tests were confirmed at 3 mos to be at an average level (including digit span, scaled score of 8: possibly the use of the English language permitted an improvement here), except for Arithmetic. Performance subtests were found to be impossible Memory was confirmed still to be impaired on the Wechsler Memory Scale, with the delayed tests of logical memory and the difficult paired associates being severely affected.

Letter recognition. Singly-presented letters of the alphabet could not be recognized correctly (1 out of 10 correct) and although it became apparent 3 mos after the accident that D.F. had recovered some word-reading ability, she has remained unable to identify letters. This failure has been found in a variety of tests, including oddity, which would exclude a naming difficulty.

Reading. Single-word reading was tested 6 mos after the accident using the Word Reading Test of the British Ability Scales, yielding a score of 48 out of 90, i.e., a reading age of 7 yrs 6 mos. Simple words like 'up', 'on', 'go', and 'he' were misread, while complex words like 'transparent', 'environment', and 'curiosity' were read correctly. Errors of misidentification were usually visual (e.g., 'tentacle' as 'rectangle', 'dough' as 'enough', and 'lethal' as 'lethargic'). The National Word Reading Test elicited 10 correct responses out of 50, giving a predicted WAIS IQ of 95; she correctly read irregular words such as 'gauche' and 'catacomb'.

Nonwords, closely matched for orthographic similarity to real words, were poorly read (4 correct out of 24 that were homophones of English words, and 0 out of 24 that were not homophones); normal readers of similar reading age (7–6) would read 63% (15) and 41% (10), respectively. In a series of tests (to be reported in more detail elsewhere), no sign has been found of differences between concrete and abstract words, nor any evidence of semantic errors. On the Schonell Spelling Test, 15 out of 50 was scored· a spelling age of 6–5. At 8 mos postaccident, some improvement on the British Ability Scales was apparent (73 correct: reading age 9 yrs 7 mos), although there was still impairment, notably on 2 and 3 letter words.

Everyday behaviour. It was noted that D.F. had no ability to recognize the faces of people familiar to her throughout the time of our testing, but she is adept at using other cues, including context and voice. She has indeed progressively improved her ability to cope with everyday activities (including shopping and cooking) over the 3 yrs that have now elapsed since the accident, though little sign of strictly visual improvement has been apparent in our tests. She has difficulty describing her visual experience, only saying that objects tend to appear 'blurred' and that separate elements 'run into each other'; words, however, are not seen as blurred, but rather as lacking structure. Although she shows no signs of aphasia, D F. has a lowered verbal fluency and spontaneity in her behaviour.

SENSORY TESTS

Visual fields

An initial attempt in Italy (at 3 wks postaccident) to carry out perimetry was unsuccessful, since D.F. was unable to see both the fixation and the target stimuli simultaneously. At 8 mos, a preliminary assessment was achieved in St Andrews, using static 500 ms presentations of a 0.5° LED; this revealed constricted fields, particularly in the upper right quadrant (from 30° outwards). Reliability was questionable due to a tendency for responses to be inconsistent.

Detection of targets defined by luminance

Achromatic displays were created on a video graphics monitor, with square patches (4 mm) of differing intensities placed as targets on a uniform grey background (2.4 cd/m^2). D.F. was asked to point to any patch she could see. The indicated patch was then removed from the screen and she was asked to find a further patch. At all contrast levels, even with Michelson contrast as low as ± 0.02, D.F. detected the patches quickly and pointed accurately to each of them.

Brightness judgements

For this task the same stimuli were used, but D.F. was asked to point to a patch and then to describe it as brighter or darker than the background grey (2.4 cd/m^2). These judgements of brightness direction were imperfect (8 or 9 correct from 10 trials) at virtually all contrast levels (target brightness levels ranged from 0.1 to 14.4 cd/m^2), and performance was highly inaccurate for a shade slightly darker than the surround (1.5 cd/m^2: contrast = 0.23). These were consistently (80%) mislabelled as 'brighter' than the surround, though always easily seen as darker by normal observers.

Eye movements

Recordings using an A.C.S. infrared sensing device showed that D.F. was able on instruction to fixate and move her eyes to and fro between 2 LEDs (15° apart, with flash durations of 0.2 or 0.3 s and an equal interflash interval), in both the horizontal and vertical planes. The saccades were made without hesitation and were accurate within the 3° amplitude resolution of the system used, showing no abnormal tendency for overshooting or for correction saccades. She moved her eyes along a piece of text when asked to read it, making horizontal saccades and fixation pauses in the normal fashion. She was also able to make tracking eye movements around the perimeter of an outline rectangle (10° × 15°) or circle (15° diameter), although the recording technique was not accurate enough to enable us to claim that this demonstrated 'implicit' access to the shape being traced.

DETAILED SENSORY INVESTIGATIONS

Visual acuity

Clinical tests of acuity could not be used because of D.F.'s inability to name letters and to report consistently the orientation of visual stimuli (*see below*). Informally it was observed that D.F. could distinguish between a grey patch and a fine dot pattern (26 dots per cm, equivalent to a resolution of 1.7 min arc).

Psychophysical detection thresholds were determined between 5 and 6 mos postaccident, using sinusoidal gratings with spatial frequencies in the range 0.625 cpd – 20 cpd. They were presented on a P31-phosphor oscilloscope screen at 35 cd/m^2, contrast being ramped on and off as a gaussian window of time (1/e = 250 ms) demarcated by a warning tone. Testing was self-paced and a yes/no (i.e., grating or no grating) one-interval procedure followed. The patterns spanned 4° of visual angle at a 114 cm viewing distance under binocular viewing conditions with head restraint, and were presented at either vertical, horizontal, or 45° orientation. A double randomly interleaved staircase method with a 50/50 protocol was used, and contrast thresholds were calculated as the mean of 5 determinations, each of which were the mean of 4 reversals on each of the 2 staircases.

As fig. 3 shows, D.F. showed a greatly impaired level of detection (by almost 1 log unit) at the lower spatial frequencies used, but appeared to detect the higher frequency gratings at normal levels. It should be noted that she tended to show perseverative responding in some threshold determinations, particularly towards the end of a test session; although these suspect determinations were repeated, the data for detecting horizontal and oblique gratings remained incomplete at 20 cpd.

Pattern evoked potentials

The P100 visual evoked potential was recorded at 15 wks from a transverse chain of occipital electrodes (each referenced to a midfrontal site) in response to chequerboard reversal (32° total horizontal extent, 50 min arc checks, 95% contrast ratio, and reversal rate 1 Hz). The P100 component was consistently prominent and bilaterally symmetric; its mean latency (108 ms) was well within the normal range, for both binocular and monocular stimulation, as was its amplitude. This demonstrates a substantial recovery

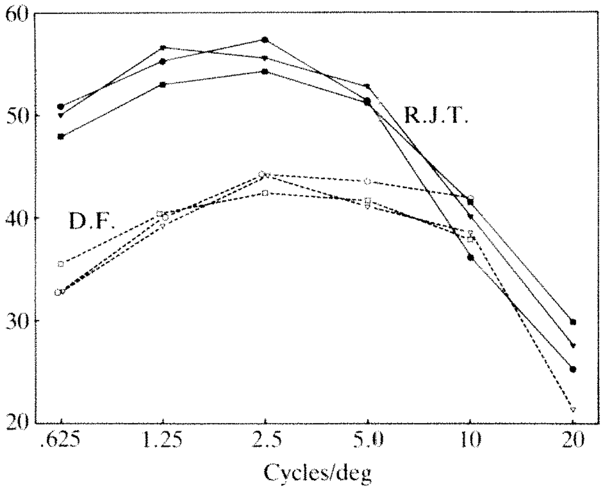


FIG. 3. Contrast sensitivity function for D.F. Sensitivity is plotted on the ordinate as dB attenuation from a Michelson contrast of 0.7. Squares denote mean thresholds using horizontal gratings, triangles, vertical gratings, and circles oblique gratings. D.F.'s data are compared with those for a matched normal control subject (R.J.T.).

from an early test carried out at Novara during week 1, when both an increased latency and a decreased amplitude had been apparent.

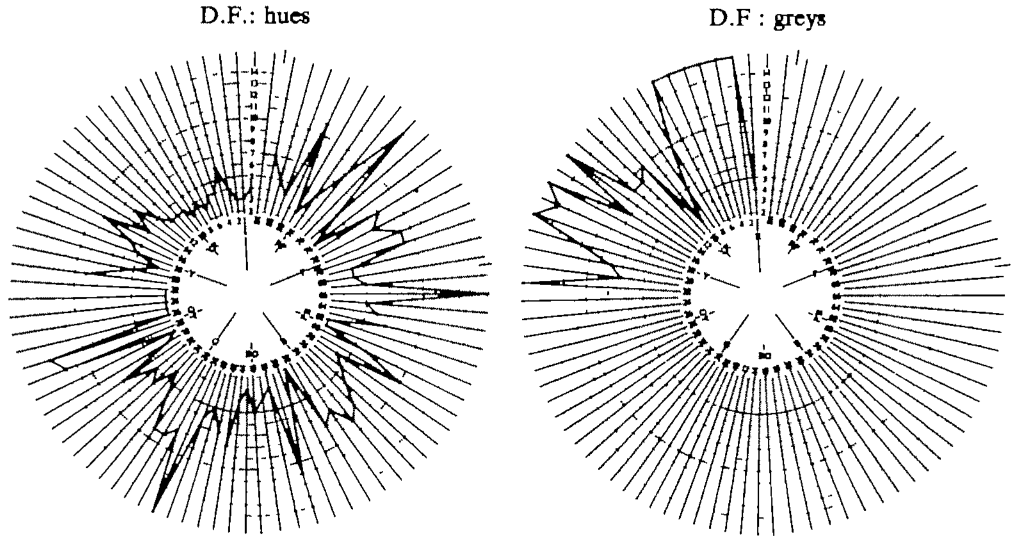
Stereoscopic depth

A series of Julesz (1971) stereograms were presented as anaglyphs for viewing through red/green filters. D.F. generally succeeded in identifying the 3-D direction of the stereoscopic depth (16/20 correct) when the dot patterns were presented at reading distance, although she was never able to recognize the shape defined by the binocular disparity. However, she failed at both direction (12/20) and shape (12/20) identification when the patterns were projected on a screen at a viewing distance of 3 m. It is possible that in these latter conditions the binocular disparity used fell below her threshold for depth perception: it was found using the Frisby stereotest that her stereoacuity was abnormally high (110 s arc as opposed to the usual ~20 s arc).

Colour and lightness discrimination

D.F. was successful in identifying most of the colours of the spots comprising the Ishihara pseudoisochromatic plates, but was never able to perceive the digit (or trace a route) that the colours depicted. The Farnsworth-Munsell 100-hue test of hue discrimination yielded scores that are outside the normal range, but which nonetheless were far better than her scores when tested for lightness (reflectance) discrimination with the equivalent Munsell achromatic (grey) series (*see* fig. 4). This superiority for hue over lightness discrimination was confirmed at 8 mos postaccident by use of the 'oddity' tests devised by Heywood *et al.* (1987), in which triplets of 7.5 cm×5 cm Munsell hue samples, or shades of grey, were presented to the subject for simultaneous comparison. For each task (2 hue sets and the grey set), there were 110 test trials. D.F. performed at a much lower level with the achromatic stimuli than with stimuli differing in hue only (Milner and Heywood, 1989). There is a notable dissociation between these results and the converse picture found in an achromatopsic patient (C.B.) tested by Heywood *et al.* (1987).

A dissociation between chromatic and achromatic channels was also strikingly apparent in a test where (9 mos postaccident) D.F. was presented with triplets of the letter 'o' (lower case typeface, 12 point), with 1 'o' in each triplet filled in. When asked to judge the 'odd' member in each triplet, D.F.'s performance was 100% correct (20/20) when the 'o's were printed and filled with red ink, but was no better than chance (9/20) when they were printed and filled with black ink (chance would be 33% correct). Repeating the same task with the 3 'o's embedded in a triplet of 4-letter words (e.g., 'lone lone lone') did not affect



D.F.: hues

D.F : greys

FIG. 4. The Farnsworth-Munsell tests D F.'s rank-ordering of sets of Munsell samples, the data being scored and presented in the standard fashion. Hue discrimination, which is relatively preserved, is contrasted with lightness discrimination, which is severely impaired.

D.F.'s identification performance (20/20 with red ink, 8/20 with black ink), even though she was unable to read the word triplets with 100% accuracy. Thus D.F. was unable to detect filled circles by use of achromatic cues, but could when additional chromatic cues were present. In a replication where triplets of circles were displayed on the screen of a graphics computer (Pluto 24i), a similar trend was observed, although it was less clear-cut: judgements were 90% correct with red lettering (37/41), and 66% with black (27/41). The difference remained significant ($\chi^2 = 5.77$, $P < 0.02$).

MOVEMENT PERCEPTION

D.F. reports great difficulty in estimating the speed of vehicles, making it impossible for her to cross a road. In initial laboratory tests it was found that she could accurately report the direction of movement of an achromatic random-dot shape against a static random-dot background, although she could not identify the shape (square or triangle) that moved. On the other hand she had an abnormality in the perception of apparent movement, such that 3 successively illuminated LEDs spaced 1° apart (flash duration 0.1 s) were seen as moving on only 75% of occasions; in these conditions normal subjects reliably saw compelling phi motion. When she did see movement, its direction was correctly identified. In a test where the 3 lights were cycled repeatedly (123123123 . . .) with a greater separation (20°), she either failed to see motion (reporting simultaneous flashes) or saw it inappropriately (e.g., as 'moving in to the centre', rather than rightward).

In more quantitative tests implemented on a Silicon Graphics IRIS 3130 computer, we used a procedure devised by Newsome and Paré (1988) in which the coherence of a moving dot pattern could be varied systematically. On each frame of an achromatic VDU screen (frame rate 50 Hz, noninterlaced) 100 dots were displayed: either 10, 25, 50 or 100% of these dots would then move in a coherent manner (up, down, left, or right), leaving the remainder (if any) to act as distractors appearing in random positions on each frame. The coherently-moving dots were displaced at a predetermined speed of 1 pixel per frame, and the dots were 5×5 pixels square (0.3° at the 80 cm viewing distance). The motion displays had a duration of 1 s and as a warning signal, the VDU flashed white 0.5 s before each display. As shown in fig. 5, at 9 mos postaccident D.F. identified the movement direction perfectly when all 100 dots moved in concert;

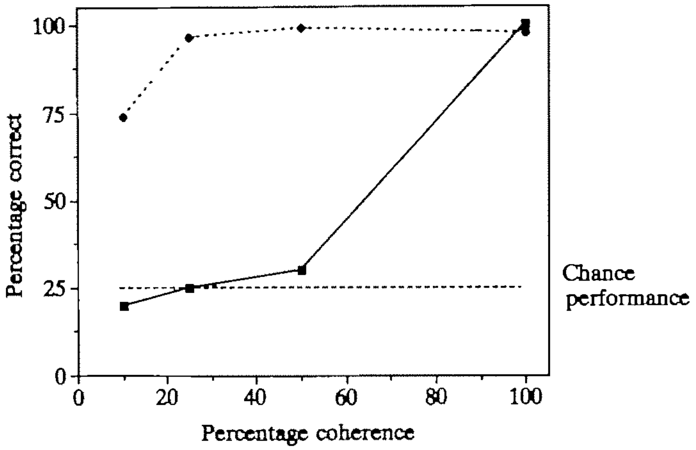


FIG. 5. Perception of motion direction. Performance in identifying the net direction of movement in a dot display in which a proportion of the elements move 'coherently' in a given direction, the remainder appearing at random locations in each frame Squares = D.F.; diamonds = 5 controls.

however, her success rate fell to chance at 50% coherence and below. Her performance remained severely impaired relative to 5 age-matched normal controls, who performed the task almost perfectly except at the lowest coherence level.

D.F. was also tested (8 mos postaccident) with videotape presentations of 'biological motion' (Johansson, 1973). The tape was prepared by first recording a person walking on a treadmill, and then frame-grabbing each of 24 successive frames constituting one step cycle and recording the x, y positions of 13 points on the body surface (head, ankles, knees, hips, shoulders, elbows and wrists). This procedure was repeated for front, left, right and back views. Using this database, walking sequences for the 4 views were generated as computer-animated dot displays (white dots on a black background). The sequences were displayed for 1 s starting at a random point in the walking cycle. D.F. could not distinguish these displays from moving jumbled arrays, created by displacing each dot in random directions at the start of the sequence by 30% of the head-to-toe height (21 correct out of 32: chance = 16/32). With a pattern exhibiting net translation as well as internal (limb) movement, however, she perceived the translation direction perfectly (16 out of 16) while remaining at chance on judgements of internal body form (normal vs jumbled, 7 out of 16).

SHAPE AND PATTERN PERCEPTION

Shape discrimination

Following Efron (1969), we used squares and rectangles matched for surface area: each stimulus was black with an area of 25 cm^2, mounted on a 21 cm×30 cm white background. As shown in fig. 6, naming performance using squares or rectangles each presented alone (in 20-trial blocks consisting of equal numbers of square and rectangle) only exceeded chance at an aspect ratio of 0.5 or less. Same/different matching of pairs of shapes (a block of trials consisted of 5 trials with 2 squares, 5 with 2 identical rectangles and 10 with 1 square and 1 rectangle) exceeded chance only at the easiest aspect ratio (0.17). Even the highest aspect ratio of 0.8 is easy for age-matched control subjects to discriminate from a square These tests were done at 5 mos.

At a later stage (15 mos postaccident) the same/different matching task was repeated using red shapes mounted on a green background, to test whether D.F.'s relatively preserved colour capacities could help her to perceive form. In the event, her scores did not improve, as shown in fig. 6. Most recently, at 2 yrs postaccident, D.F.'s performance with black shapes still remained virtually unchanged (not shown in fig. 6). Performance was poorer still (a mean of 67% correct across the 3 easiest rectangles) when the shapes

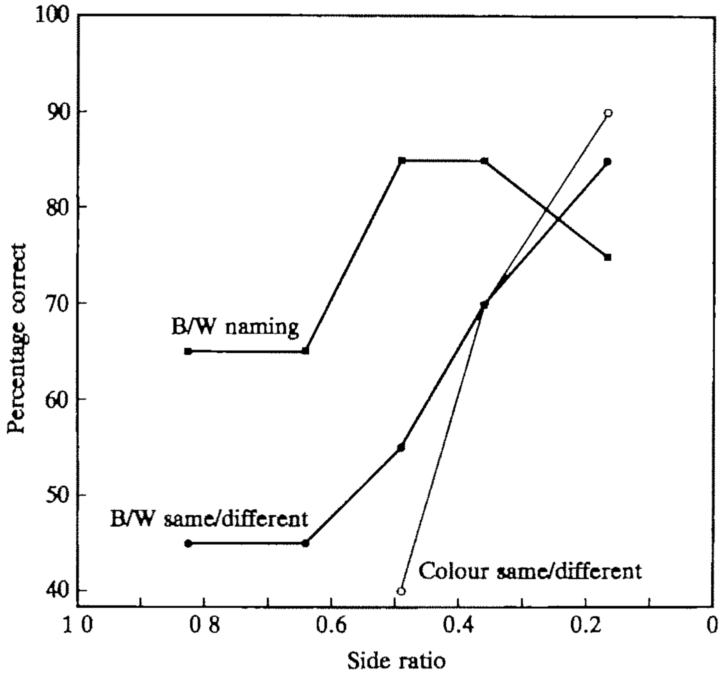


FIG 6. Discrimination of shape: the Efron task. Percentage correct performance under 3 different test conditions solid squares = naming of a single black shape (square or rectangle); solid circles = same/different judgements of 2 adjacent black shapes, open circles = same/different judgements of 2 adjacent coloured shapes. All data points are based on at least 20 trials.

consisted of square and rectangular patches of random dots (Letratone LT98 on white paper) moving over an identical random dot background.

Similar difficulties were apparent in matching-to-sample tests using 4 black filled shapes (either meaningful silhouettes of animals, or using the geometrical figures rectangle, trapezium, parallelogram and diamond; *see* fig. 7F), performance was at chance (25%). Consequently, there are impairments of shape discrimination where the shape is defined by luminance, colour or motion, as well as by binocular disparity, as noted earlier.

Shape detection

In an adaptation of the test devised by Warrington and Taylor (1973), simple figures (+, O, X) were presented either at 100% contrast against its background or noise-masked by reversing the contrast of 25% of the pixels (i.e., 75%/25% contrast, or vice versa). Examples are given in fig. 7A. D.F. reported the figure's presence (though not its identity) on all of the 100% contrast trials, but was at chance on the masked trials (12 correct out of 24).

Symmetry perception

Perception of the symmetry or asymmetry of abstract shapes was tested at 8 mos using figures composed of 3 or 4 geometric elements combined in vertical stacks which were then divided at the vertical midline and recombined in different ways (*see* fig. 7B). With stimuli subtending 10° × 5° at a viewing distance of 40 cm, performance was at chance (8 out of 16). The test was then repeated using smaller patterns (3.5° in height), either black or red, on white backgrounds. Black patterns gave chance (50%) performance (11/24 and 6/12), while red ones gave a temporary hint of improvement (19/24 but then 6/12).

Circular dot arrays with an 8-fold pattern of radial symmetry (*see* fig. 7C) were also used: symmetry

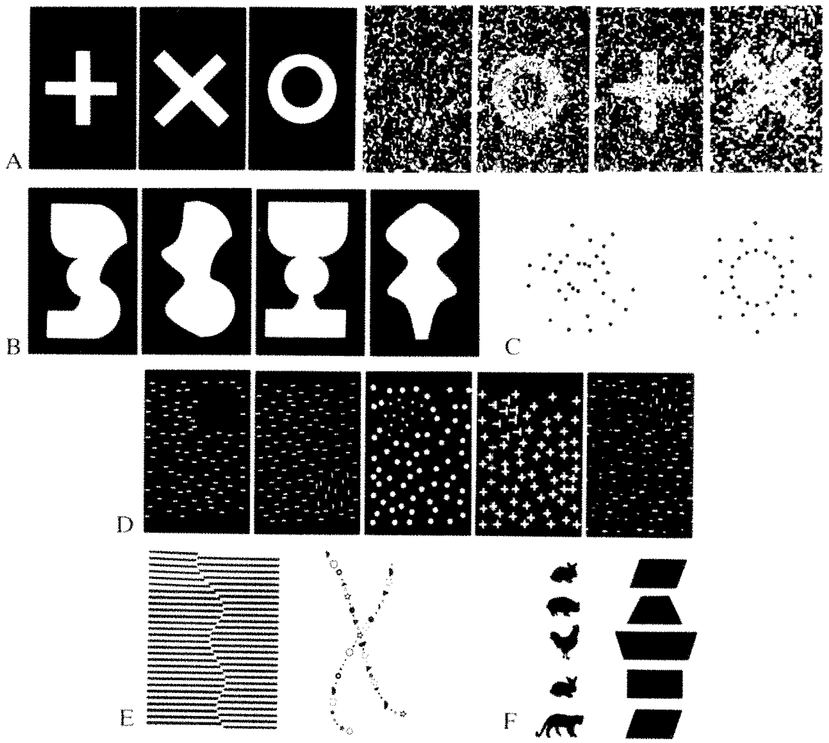


FIG. 7. Visual stimuli used for various perceptual tests. A, shape detection task. From left to right: target shapes at 100% contrast, and shapes (with a no-shape control) in which the direction of contrast of 25% of the pixels has been randomly complemented. Adapted from Warrington and Taylor (1973). B, C, stimuli for assessing symmetry perception. B, symmetry about a vertical axis. C, asymmetric pattern and radial pattern with eight-fold symmetry. D, stimuli for assessing texture perception. From left to right: texture differences defined by intensity, orientation, size, texton differences, and orientation with size as an irrelevant cue. E, stimuli used to test perception of continuity. Wavy lines defined by the continuity of vernier offset of grating elements (*left*) or pattern elements (*right*). F, shapes used in matching to sample tasks. *Left*: meaningful silhouettes (animals). *Right*: geometric shapes.

judgements were at chance (6/10 correct), as were judgements of attractiveness (more flower-like), giving no evidence for any 'implicit' appreciation of symmetry (6/10 correct).

Line orientation

In tests given at 4 mos postaccident, square-wave gratings of different spatial frequencies were presented at high contrast with either a horizontal or vertical orientation. D.F. scored close to chance both with the use of a coarse (0.14 cycle/deg) grating (7/16 correct) and with a 1.1 cycles/deg grating (5/12). Occasionally following a verbal error a check was made that she was using the words correctly, by asking her to signal the judgement by orienting her arm; this action always corresponded perfectly with the verbal report.

Since she was able to use the 'oddity' principle to demonstrate relatively good hue discrimination (Milner and Heywood, 1989), D.F. was given a similar test of orientation discrimination at 9 mos. On each trial 3 circles (4.5 cm diameter) were presented on the table, each containing an achromatic high-contrast square wave grating (0.5 cm bar width), 2 at the same and 1 at a different orientation. The orientation of the stimuli varied between 10° and 90°, and the difference between them on a given trial was either 45, 67.5, or 90°; 32 trials were given. Success at indicating the odd stimulus was significantly above-chance at 90°

(70% correct), but fell to chance for smaller angular differences (37% correct: chance = 33%).

D.F.'s ability to judge the orientation of a large open slot was also severely impaired (*see below*).

Dot numerosity

At 3 mos D.F. was asked to state the number of high-contrast spots presented on a sheet of paper. When only 1 was presented, performance was perfect, but with 2 it fell to 33% correct, and with 3–5 spots it fell to 0%. Although there was a significant correlation across the 25 trials ($r = +0.68$, $P < 0.01$) between the number presented and the number reported, D.F. never reported seeing more than 3. This failure held for arrays both of large (10 mm diameter) and of small dots (1 mm). The test was repeated at 22 mos postaccident, using 4 mm dots (either black or red) placed in a cluster spanning up to 10 cm on A4 sheets. Performance was now almost perfect for 0–3 dots, whether tested with black (19/20 correct) or with red dots (20/20), but between 4 and 6 dots, scores fell to a mean of 10% correct (3/30) for both colours. These difficulties cannot be attributed to an impairment in counting per se since D.F. was faultless at counting up to 6 auditory taps made out of sight at an irregular rate (0.2–1.0 Hz).

Texture differences

A number of photographs of 'random' patterns were made, some of which contained an area (approximately one-eighth of the total) where there was a change in the texture. The task was to say whether there was an area that was different from the rest of the photograph, and then to point to the area and describe how it differed. Unlimited time was given for each presentation Texture differences included (1) an area of missing texture elements (therefore constituting a luminance difference), (2) a 90° orientation change, (3) a 2-fold linear size change, (4) a micropattern or texton difference (Julesz, 1981), and (5) an orientation difference where size was made an irrelevant and distracting cue. Examples are given in fig. 7D. Stimuli were presented as 15 cm high-contrast black/white prints or as back-lit 35 mm slides at a viewing distance of 30 cm. A follow-up test was given (14 mos postaccident) using video displays of equivalent patterns that were black on white, white on black, red on black, and red on white.

D.F. could not discriminate homogeneous textures from textures containing a distinct patch with any of the 5 available cues. This was true with 2-choice discrimination and with a single area to find in one stimulus. The same failure was observed on 2 occasions separated by 6 mos. Furthermore, the size and colour of the stimuli presented did not make any difference to performance. D.F. would often detect an area that appeared to her different in an inappropriate part of the display and would often describe the difference in texture wrongly, for example, claiming an orientation difference where a size difference or texture absence was displayed. (It may be noted that D.F.'s failure to perceive form from colour in the Ishihara plates may have been secondary to a segmentation deficit of the same kind as that apparent with these other textural qualities.)

Gestalt perception

As well as being unable to perceive Gestalt grouping by similarity in the texture stimuli just described, D.F. could not perceive grouping by contiguity; for example, she saw large letters composed of small (different) letters only as unstructured sets of small letters (usually themselves misidentified). Similarly, strings composed of nonverbal symbols (stars, dots, etc.) were never seen as strings, although individual elements could often be identified (fig. 7E, right).

Vernier offset and grating continuity

D.F. could not identify (verbally or by pointing) a vernier offset in a single line (0.7 mm wide) even at a maximum displacement of 0.35 mm. She was presented with a related task in which a break in parallel lines traced a curved contour (e.g., fig. 7E, left): this could be seen as testing also whether there was any perception of grouping by continuity. Whereas D.F. noted the presence of a break correctly in 5 out of 6 presentations, she was unable to point to or trace the correct line of breakage, in one instance tracking a line almost 90° to the vernier break. In a retest (13 mos postaccident) with similar stimuli created on a Pluto 24i graphics system in black on white, red on white or red on green, D.F. could not correctly identify the position or continuity of line breaks using either intensity or chromatic differences, or both

Topographic imagery

D.F. was asked a number of questions that required her to visualize a particular area—her mother's kitchen and living room, and 1 of the 3 main streets in St Andrews (South Street). For the home rooms she was asked to say where items (e.g., TV, kettle, coffee) were kept. For the street she was asked what could be seen if looking East from a stated standpoint. D.F. gave responses quickly and confidently to these questions, and her accuracy was independently validated.

VISUOMOTOR TESTS OF ORIENTATION DISCRIMINATION

Despite D.F.'s poor performance on shape recognition tasks, she had little difficulty in everyday activity such as opening doors, shaking hands, walking around furniture, and eating meals. It was further observed in informal testing that she could accurately reach out and grasp a pencil orientated at different angles. These observations suggested that D.F. might have some residual visual contour and edge information available to her for the visuomotor guidance of action, even though it was not accessible to her perceptual awareness. In pursuing this possibility, we used a modified version of the technique devised by Perenin *et al.* (1979), in which the subject is required to reach out and place the hand into a rectangular slot held at different orientations.

Experiment 1

As shown in fig. 8, D.F. was presented with a 12.5 cm×3.8 cm slot cut into a white disc, which on different trials was orientated at 0° (vertical), 45°, 90°, or 135° Raising the hand from the resting platform started a digital clock, which was video-recorded along with the movement of the hand towards and into the slot, by means of 2 cameras; 1 overhead and 1 at the side. Measurements of the horizontal and vertical extents of the hand across the palm were made from still frames of a split-screen videotape at 100 ms intervals, and hand orientation was calculated at each point of the trajectory. Both hands were tested for 8 trials at each orientation, at 5.5 mos postaccident. As shown in fig. 9, D.F. correctly matched her hand orientation to that of the slot during the course of the movement, well in advance of contacting the target (which was reached at about 750 ms). Yet verbal judgements in the absence of reaching, on an equivalent series of slot presentations, were made at only 55% correct (chance = 25%).

Experiment 2

In a second test session 1 wk later, reaching movements and orientation judgements were again both tested. For the former, an 8 cm×8 cm card was held in the right or left hand and 'posted' into the slot on each trial; this allowed more accurate measurements to be made from the videotapes. The judgements

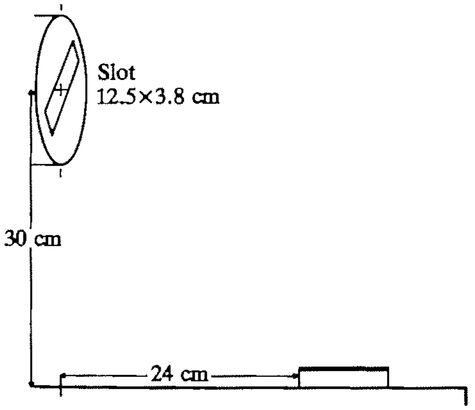


FIG. 8. Reaching task. The subject was asked to move the hand towards and into the slot, starting from the resting plate (holding down a microswitch) on each trial.

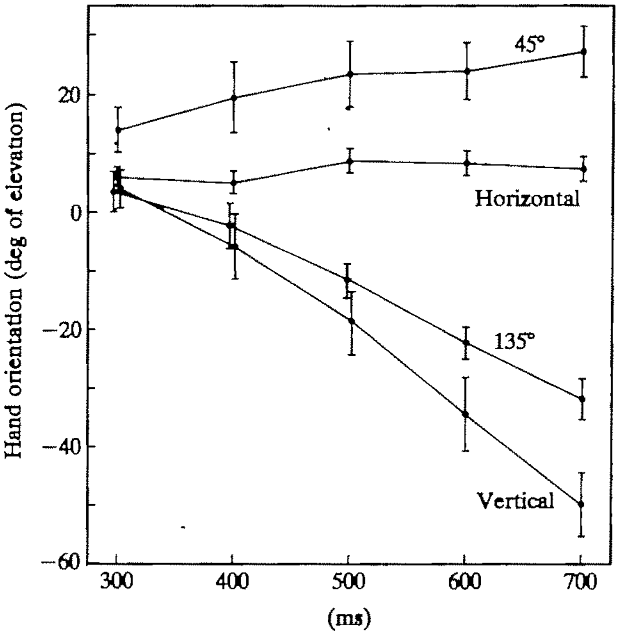


FIG 9 Visuomotor coordination: Experiment 1. Hand inclination (averaged across left and right hands) during reaching towards the orientated slot. The error bars show the SEM.

of orientation were elicited nonverbally, instead of being spoken as before; it was hoped that this would reduce ambiguity, particularly in relation to the 45°/135° judgements. The task was to match the orientation of the target slot on a series of trials by manually rotating a second slot cut in a disc that was mounted inside a 20 cm×20 cm box and had to be perceived tactually. The results for reaching are shown in fig. 10, which clearly demonstrates accurate orientation of the card during the reaching trajectory, well ahead of arrival at the slot (which occurred at about 930 ms). In contrast, as shown in fig. 11, D.F.'s manual matching of the slot's orientation was no better than chance ($P > 0.05$ for all 4 orientations tested). These judgemental data are presented as polar diagrams together with those of 2 normal age-matched subjects who both show a high level of matching accuracy.

Experiment 3

Fourteen mos postaccident (8 mos after Experiments 1 and 2), D.F. was retested on the hand-reaching task, first using the previous white disc and secondly using a grey disc (expected to reduce reaching accuracy by reducing the luminance contrast between the disc and the slot). Six trials were given at each orientation, using only the preferred right hand. Manual matching judgements were again tested, but now the subject adjusted a rotatable slot which was placed in full view in front of her on the table. Matching was tested separately for the white disc, the grey disc, and also with a green disc (as a possible way to improve matching performance, given D.F.'s spared colour discrimination ability). Performance in this visual matching task was now slightly better than chance ($P < 0.01$) with the white disc (either because of some recovery or because no cross-modal or tactile-discrimination element was now present in the task). However, it reverted to chance ($P > 0.05$) with both the grey and the green discs, presumably because in both cases there was a lower luminance contrast, and no benefit to be gained from a chromatic difference.

In contrast, hand orientation during reaching continued to anticipate the target orientation accurately for both target discs. Fig. 12 shows the judgemental settings for the grey disc, together with the corresponding final orientation of the hand in the reaching task (at the instant the hand arrived at the target). This method of analysis allows a quantitative comparison of performance accuracy in the 2 tasks: SDs are given

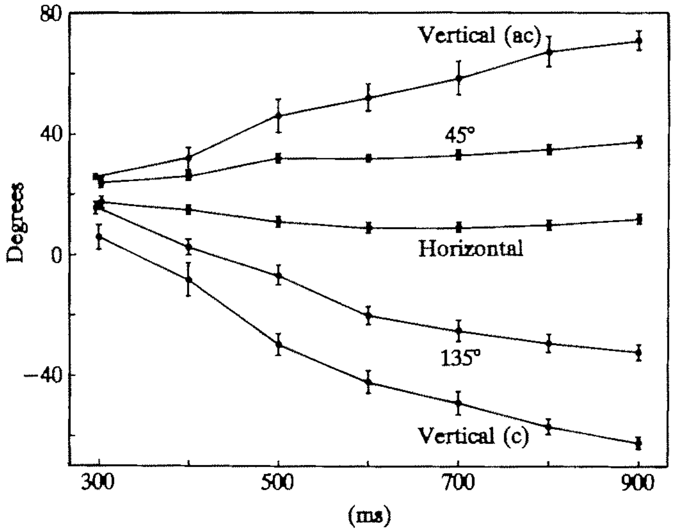


FIG. 10. Visuomotor coordination Experiment 2. The inclination of a card as it was moved towards the slot to be 'posted'. The data are again averaged between left and right hand tests. Occasions when D.F. rotated the hand clockwise (c) and anticlockwise (ac) whilst approaching the vertical slot are presented separately

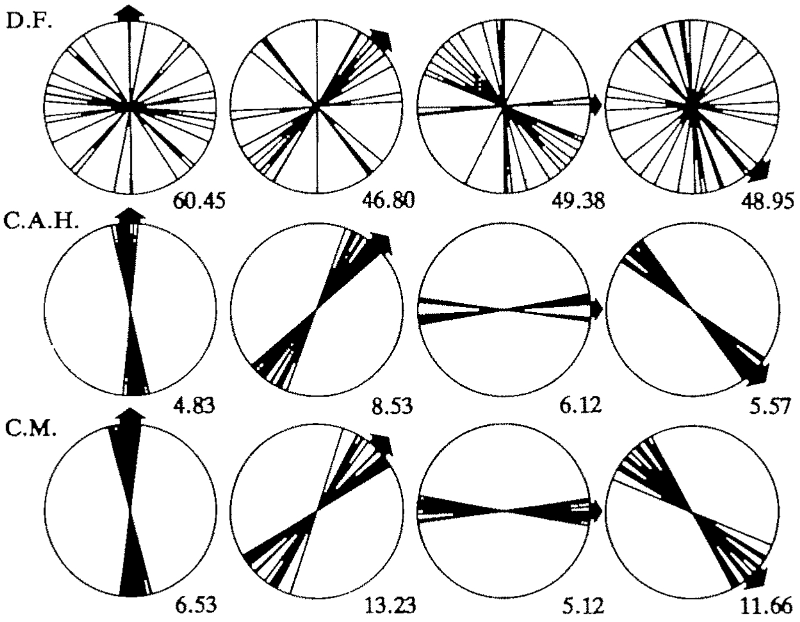


FIG 11. Orientation judgements: Experiment 2 Polar diagrams representing the manual settings made to match a visible slot orientated at 0°, 45°, 90° or 135°, shown respectively from left to right. The data for D F. are contrasted with those of 2 normal controls matched for age. SDs of the settings are given numerically, in calculating these it was assumed that all responses were within 90° of the true orientation.

numerically below the polar diagrams. Variance ratios between the corresponding tasks were in fact of a similar magnitude for the 2 discs, and were highly significant, vastly beyond the 0.001 level at all orientations except the vertical ($P < 0.01$ for both discs). Thus it is clear that with both discs there is a high level of accuracy in orienting the hand during reaching towards the slot, contrasting markedly with a low judgemental accuracy in the matching task.

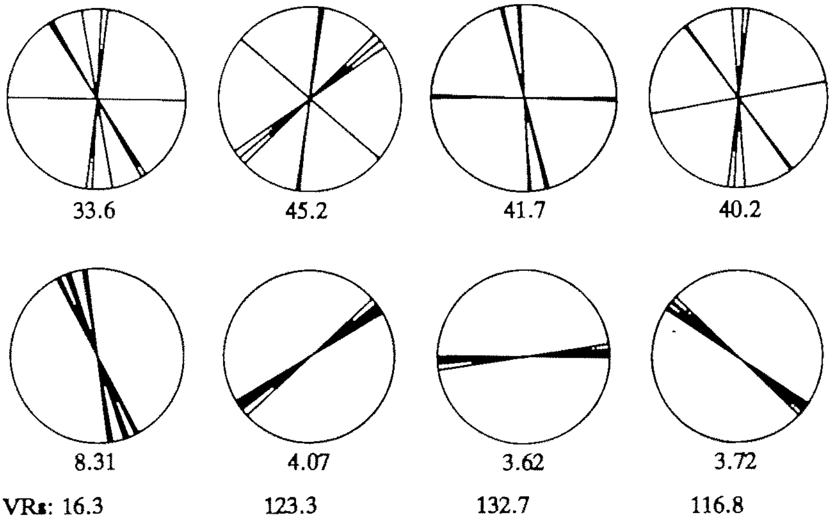


FIG 12 Accuracy of reaching and judgement: Experiment 3. These polar diagrams compare the accuracy of judgements made manually by rotating a visible matching slot (*top*) against terminal hand orientation in reaching (*bottom*) The 4 target orientations used are shown across the figure (vertical, 45°, horizontal, and 135°, respectively). Data are shown for the grey target disc. Only the right hand was used throughout Experiment 3. VRs = Variance ratios

Summary

These 3 experiments document a well-preserved capacity to coordinate motor behaviour in direct relation to the orientation of a visually presented object. Despite this, they also show that D.F. was unable to make accurate perceptual judgements of slot orientation, whether expressed as verbal descriptions or as manual matches with either tactile or visual feedback. The dissociation thus appears not to be between the use of verbal versus manual response channels; instead it seems to be between conscious perceptual judgements and the more 'automatic' visuomotor guidance of a skilled action.

DISCUSSION

The lesion

D.F. inevitably sustained diffuse brain damage as the result of the carbon monoxide poisoning that she suffered. This may be illustrated by her early amnesic difficulties, perhaps reflecting damage to medial temporal lobe structures, and also by a loss of spontaneity and verbal fluency, which may reflect frontal damage. Her acalculia and apparent decline in some aspects of verbal intelligence and short-term verbal memory (though there are no prelesion data for comparison) may reflect left parietal damage. The EEG showed bilateral abnormalities, in keeping with diffuse anoxic cerebral damage, though most prominent posteriorly and in the temporal regions.

It is striking, however, that the major abnormalities in the later MRI studies were restricted to the occipital poles bilaterally, the damage extending laterally in the ventral part of the occipital lobe (area 18) and dorsally in the posterior parasagittal occipitoparietal region. There was little indication of any abnormality in area 17 medially, except possibly for a small spot in the left calcarine sulcus. (It is notable that during the early stages of the disease process, when visual disability was most pronounced, neither CT scanning nor MRI clearly revealed the cortical damage, whereas SPECT did indicate an impairment of occipitotemporal function, at least on the left.)

The nature of visual form agnosia

The primary intention of the present report is to attempt an understanding of D.F.'s visual difficulties, especially the failure of shape perception which appears to be the root cause of her object recognition loss. It is clear that D.F.'s visual deficits are severe, and that they affect a wide range of sensory and perceptual functions, including brightness, motion, depth, and shape perception. This complex pattern of deficits is unlikely to be completely reducible to one or two readily enunciable factors. However, it is of less interest to catalogue all the possible effects that such devastating brain damage might have than to examine the dissociations seen between particular tasks that have much in common with each other. For example, it may be that D.F.'s visuospatial span of attention is reduced, causing difficulty in our tests of dot enumeration; but it is not clear that such an attentional disorder could affect performance differentially in oddity tasks, such that hue differences can be distinguished but reflectance differences or orientation differences can not. Nor could it explain the fact that words can be identified under conditions where the direction of their luminance contrast cannot be. In short, attentional and other generalized difficulties may be compounded with, but cannot explain, the perceptual losses we have documented.

In order to identify a particular shape, the brain must determine the orientation of the component boundaries. To do this it can use many different visual submodalities, including intensity, colour, saturation, stereopsis, and motion (through shear, occlusion, or coherence). It is therefore necessary to conceive of edge orientation, not just as a primary quality extracted by luminance-based 'edge detectors', but rather as a higher-level attribute. D.F. failed to perceive boundary shape or orientation accurately whether conveyed by colour, intensity, stereopsis, motion, proximity, good continuity, or similarity: thus edge perception may be disrupted at a high level. She was also unable to segment perceptually part of a pattern on the basis of common textural properties of size, orientation, intensity, or shape (e.g., + vs T) of its component elements. Such texture discrimination does not require judgements of boundary orientation or shape per se. Perceptual grouping based on similarity in size and intensity would thus seem to be deficient independent of deficits in orientation processing. There therefore seems to be a generalized inability to perceive shape and 'shape primitives', that is, basic figural qualities that would serve in the recognition of many different objects. These would include the Gestalt grouping principles (proximity, closure, similarity, good continuity and common fate) and also other descriptors such as parallelism (Biederman, 1987; Lowe, 1987).

We would argue that D.F.'s form-recognition deficit derives not from a loss of particular input channels ('edge detectors') but rather from a deficit arising at a higher level, at or beyond a general purpose mechanism for extracting shape primitives. Yet it seems that some information about boundaries and shape primitives must be available to certain other brain mechanisms in order for the patient to be able to perform tasks such as manual prehension and word recognition. Therefore it is proposed below that mechanisms of *object perception* (but not all processing systems) may be selectively disconnected from such an early shape processor. But in addition, D.F. has other deficits (e.g., a detection failure selective for low spatial frequencies) that it would seem implausible to attribute to a partial disconnection: hence an additional factor has to be postulated.

Loss of the 'magnocellular stream'?

It was speculated in a recent report (Milner and Heywood, 1989) that at least some of the deficits seen in D.F. could be understood in terms of a selective lesion to the *magnocellular visual pathway*. This refers to a subdivision of the visual system that originates in the alpha ganglion cells of the retina, includes the magnocellular layers of the dorsal LGN and layers 4C-alpha and 4B of the striate cortex (V1), and thence projects to the 'thick stripes' of the adjacent area V2 (Livingstone and Hubel, 1988; Zeki and Shipp, 1988). The properties of cells in this system suggest that it is involved in the analysis of low spatial frequency pattern processing and in depth and motion processing, and that it is spectrally broad-band or 'colour blind'. Damage to it might be expected to lead to several of the deficits seen in D.F.: specifically, to reduced depth and motion sensitivity, a loss of detection sensitivity at low spatial frequencies, to impaired figure/ground segregation, and perhaps also to impaired brightness discrimination (Livingstone and Hubel 1988). In contrast, higher spatial frequencies would not be expected to be affected, nor would colour perception.

It is not implausible to suppose that one or another part of this 'magnocellular' system might be selectively vulnerable to anoxia or toxic influence. However, it seems unlikely in the present case that the critical damage is at a level before V1, since measures of the occipital P100 component of the evoked potential to reversing chequerboard patterns recovered to normality. Particularly if the P100 wave is primarily mediated by the magnocellular pathway, as suggested by its spatial frequency response characteristics (Previc, 1988), then that system would appear to be intact in D.F., at least up to V1.

It is notable that the only previous patient of this type to be tested for psychophysical contrast thresholds also revealed a reduced sensitivity for lower spatial frequencies (Abadi *et al.*, 1981), consistent again with a loss of the 'magnocellular' system. The visual evoked potentials in that previous patient (R.C.), however, showed a reduced amplitude to a low spatial frequency (1 cpd) grating under pattern-reversal conditions. R.C. may therefore have suffered damage earlier in the system than did D.F.

It is proposed then that there is a selective lesion of the magnocellular stream at the early cortical level (e.g., a lamina-selective lesion in V1 and/or a thick stripe selective lesion in V2; *see* Livingstone and Hubel, 1988). However, this alone cannot provide a convincing account of D.F.'s shape discrimination loss, because her good acuity suggests that the 'parvocellular' processing stream, which runs in parallel to the magnocellular system, is relatively intact. This stream (more precisely its 'parvo-interblob' branch; Livingstone and Hubel, 1988) contains cells at the V1 level that are orientation-selective at high spatial frequencies. The problem therefore remains as to why shape discrimination should be lost, given that an intact 'parvocellular' system could potentially provide the necessary pattern information.

It should be noted in parenthesis that D.F.'s explicit awareness (and reasonably good discrimination) of colour may be attributed to a sparing of the *third* stream that passes through V1, the cytochrome oxidase-rich 'blob' stream. In this system cells are wavelength-coded but have virtually no orientation selectivity (Livingstone and Hubel, 1984).

A disconnection hypothesis

D.F. was able to modify the posture of her hand to match the orientation of a slot towards which she was reaching, yet she was unable to *perceive* the orientation of the slot. This appears to add a further instance of 'implicit' knowledge in the absence of 'explicit' knowledge, to those that have been documented for several other neurological conditions (Schacter *et al.*, 1988). She was impaired both in reporting the orientation verbally and in visually or tactually matching the orientation using a second slot. Moreover, her performance in other tests of orientation perception (e.g., oddity tasks) was consistently impaired. In more recent studies (Goodale *et al.*, 1991) we have extended this finding and shown that D.F. can also demonstrate implicit knowledge of the size and orientation of solid rectangular objects when asked to reach and grasp them.

Failures of orientation discrimination are not unusual following posterior right hemisphere damage (e.g., De Renzi *et al.*, 1971; Benton *et al.*, 1978), although they would be unlikely to cause an object or shape-recognition deficit. Undoubtedly it would be of great interest to test whether any such patients can, like D.F., demonstrate good implicit processing of contour orientation in visuomotor tasks. Likewise, it would be interesting to examine the visuomotor processing of orientation information in previously described 'dysmorphopsic' patients, some of whom are known to be able to reach accurately towards point targets in localization tasks (Warrington, 1985*b*).

It is proposed that D.F. can process many perceptual attributes (including luminance contours and brightness levels), but that the output routes to brain systems concerned specifically with recognition have been interrupted. Specifically, the disconnection might block the output from areas V1 (and perhaps V2) of the surviving form-related (parvo-interblob) system en route to higher processing systems in the temporal neocortex (presumably by way of the human equivalent of area V4: Maunsell and Newsome, 1987; Zeki and Shipp, 1988). But in D.F. this disconnection would be in addition to a hypothesized disruption within the cortical magnocellular system. Therefore, it must be assumed that other outputs of the cortical parvocellular system remain intact, making possible D.F.'s ability to orientate her hand in reaching towards an orientated visual slot, and her equally remarkable ability to identify many words.

A striking double dissociation may be noted between D.F. and the patients with optic ataxia recently described by Perenin and Vighetto (1988). Those subjects showed a converse impairment, in being able to judge the orientation of a visual slot but not to use such orientation information to guide their hand movements towards and into the slot. Their lesions, predominantly in the parietal lobe, evidently did not interfere with the *perception* of orientation, and none of the patients had agnosic problems; but the lesions severely disrupted visuomotor coordination in respect of stimulus orientation and spatial location. It may be surmised that the parietal mechanisms disrupted in optic ataxia, and their visual inputs, are largely intact in D.F. The MRI localization evidence that her lesion spares the dorsolateral parietal area, and most of V1, would be consistent with this view; and it is known that there is a direct input from V1 to the parietal cortex in the monkey (Colby *et al.*, 1988).

A different disconnection might explain the unusually clear dissociation between D.F.'s failure to name or match letters and her relatively good word recognition. While this type of dissociation is not uncommon in patients exhibiting the symptom complex of deep dyslexia (e.g., Howard, 1987), it is unusual outside that complex. Indeed, we have found no evidence that D.F.'s difficulty in reading is anything other than perceptual in nature. She is unable to perceive individual letters, just like other simple visual shapes. Yet remarkably, visual information is able to access mechanisms for the recognition of word form. This dissociation clearly contrasts with those patients with pure alexia who can only read letter-by-letter (and who often have no disorder of object or face recognition (Damasio and Damasio, 1983). Our preliminary speculation would therefore be that although visual pattern information is extensively processed in D.F., it is disconnected from the process of letter recognition. The information must, however, retain access to a word-form recognition process that is apparently inaccessible in letter-by-letter readers. It is interesting that this word-form access seems, like D.F.'s visuomotor performance, to be achieved without conscious mediation: she says that she is 'guessing' the words. A more detailed discussion of D.F.'s letter and word recognition is beyond the scope of the present paper, and will be published elsewhere.

D.F.'s impaired ability to discriminate lightness differences while able to discriminate hue differences has been discussed previously (Milner and Heywood, 1989), and led in part to the hypothesized loss of 'magnocellular' processing in D.F. These findings have been extended here by our finding that D.F. can detect small differences in luminance despite being unable to assign the correct contrast direction. Furthermore D.F. can use luminance information for reading, even when unable to discriminate the contrast direction of the words against their background. Although the disconnections postulated above would apply most readily to the perceptual disorders of form and pattern, it is possible that the arguments could be extended to suggest that outputs from the central mechanisms for brightness processing are also differentially spared in D.F. Alternatively, however, it is possible that her ability to detect and use brightness differences but not to judge their direction, could stem from a reliance upon an intact subcortical (tecto-pulvinar-parietal) route to the cortex. This route may permit difference detection but not directional discrimination.

Although the generality of our findings may be limited, it is interesting that a preservation of visuomotor skills was reported in two earlier accounts in the literature. The patients studied by Goldstein and Gelb (1918) and by Landis *et al.* (1982) were both unable to perceive visual form, and therefore would probably fall into the category of 'visual form agnosia'. Both patients were, however, able to trace the contours of letters and forms by moving their finger or head in a trajectory matched to the target outline. Indeed, the patients were so successful at this tracing that they could introspectively monitor the proprioceptive feedback from the tracing movements and thus infer the identity of a form, letter, or word. When tracing movements were disallowed by the experimenters, visual recognition of shapes, letters and words became impossible.

This dissociation in the patients of Goldstein and Gelb and of Landis *et al.* might be interpreted in much the same way as those observed in patient D.F.: direct routes from visual boundary analysis to pattern recognition mechanisms may have been blocked. The preserved visuomotor coordination required for tracing may be postulated to depend on routes from the analysis of visual contours in the occipital lobe to parietally-located systems controlling finger and head movements. Nonetheless, it should be noted that D.F. differs from the cases of Goldstein and Gelb and of Landis *et al.* in being unable to trace manually the visual shape of letters or shapes, and in any case not needing to trace the shape of letters in order to read words.

Previous models of visual form agnosia

Two previous proposals were mentioned in the Introduction: a generalized failure of 2-D form analysis (Warrington, 1985*a*; Humphreys and Riddoch, 1987*b*), and the hypothesis of Campion and Latto (1985) involving 'peppered scotomata'. We would agree with the former authors that a failure of '2-D sketch' formation could explain the failure of some 'form agnosic' patients on shape discrimination tasks like that of Efron. However, such a failure should disrupt all form processing. It is therefore difficult to see how it could account for the dissociations reported here between successful rotation of the hand in reaching towards a slot versus unsuccessful explicit report of its orientation; or between adequate word reading versus failure in letter identification. We would argue therefore that the impaired shape perception of our particular patient cannot be understood in this way.

Campion and Latto's hypothesized 'peppered scotomata' would likewise disrupt all shape processing, and other visual processing too. However, it fails to account for the dissociations we have found between the hue and the lightness versions of the Farnsworth-Munsell test or the Heywood *et al.* oddity task, and between detecting or using luminance differences and assigning contrast direction to them. It also has several other problems. It would predict that visual impairments should be related to the size of the stimuli, with stimuli small enough to remain within an intact region of visual space, or large enough to span several, being relatively spared. There was no evidence of such a size effect, for example, in our tests of dot enumeration, orientation, or texture perception. Nor was there any evidence that the use of scanning eye movements was especially beneficial to D.F. (she was given unlimited time in most tests). Yet on Campion and Latto's hypothesis, scanning movements should be of great benefit, since stimuli could be brought onto intact areas of the field by this means. It might also be noted that there is no sign on MRI of any disseminated lesion either in the calcarine cortex or in adjacent cortex (where the lesion seems rather continuous) in our patient; however, it could no doubt be argued that the images are of insufficient resolution to show this hypothetical pattern.

It must be freely confessed that our own suggestions do not satisfactorily explain all the intriguing dissociations that have emerged from our investigations of D.F.; they are offered merely as a beginning in unravelling the previously unsuspected complexities that exist in at least one patient with 'visual form agnosia'.

ACKNOWLEDGEMENTS

The authors are grateful to C. P. Cowan, M. H. Harries, M. Oram, M. D. Rugg, P. H. Silver, S. Thomas and R. J. Thomson for their help in testing and in test construction. They also thank Professor G. Ettlinger and Dr R. C. Roberts for their comments on a draft manuscript, and Dr M. Campbell for suggesting the term 'dysmorphopsia'. They are deeply indebted to D.F. and her family for their patience and cooperation. A preliminary communication of some of the results reported here was presented by A. D. Milner to the Experimental Psychology Society, Swansea, April 1989.

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(Received October 13, 1989. Revised February 20, 1990. Accepted March 6, 1990)

Brain (1991), 114, 429–442

CHRONIC RELAPSING EXPERIMENTAL ALLERGIC NEURITIS INDUCED BY REPEATED TRANSFER OF P_2-PROTEIN REACTIVE T CELL LINES

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SUMMARY

Chronic relapsing experimental allergic neuritis (crEAN) was induced by repeated transfers of P_2-protein reactive T lymphocyte lines. Clinically, each intravenous transfer of P_2-reactive T cells induced a relapse of the disease with weight loss and flaccid paresis of the hindlimbs followed by recovery. After multiple transfers, recovery from disease was incomplete, leading to increasing neurological deficit during the remissions. The pathology of the lesions during exacerbations was characterized by massive inflammation in the peripheral nervous system, associated with extensive endoneurial oedema, nerve fibre destruction and wallerian degeneration. Selective primary demyelination and remyelination was found in the minority of affected nerve fibres. No onion bulbs were present in chronic lesions. In the central nervous system partial degeneration of the posterior columns reflected the extent of wallerian degeneration in the peripheral nerves and spinal roots. In addition, during stages of active disease some T lymphocytes and upregulation of Ia antigen expression were found in the spinal cord.

INTRODUCTION

Experimental allergic neuritis (EAN) is an autoimmune disease, induced by active sensitization with peripheral nerve tissue (Waksman and Adams, 1955) or with purified components of peripheral myelin such as P_2-protein (Kadlubowski and Hughes, 1979), P_0-protein (Milner *et al.*, 1987), galactocerebroside (Saida *et al.*, 1979) or gangliosides (Nagai *et al.*, 1976). Pathologically, EAN is an inflammatory demyelinating disease of the peripheral nervous system. The extent of demyelination, however, is variable from model to model and is most pronounced in chronic variants of the disease (Wiśniewski *et al.*, 1974; Craggs *et al.*, 1986; Harvey *et al.*, 1987; Adam *et al.*, 1989).

Both cellular and humoral immune mechanisms appear to be involved in the pathogenesis (Saida *et al.*, 1987). Recently, it was demonstrated that a T cell population, which reacts specifically against the P_2-protein of PNS myelin, can be isolated from rats immunized against PNS homogenates (Linington *et al.*, 1984). Such T cell lines very efficiently transfer EAN to syngeneic animals. Thus a model was established which defines the immunological and pathological effects of autoaggressive T lymphocytes within this PNS disease (Izumo *et al.*, 1985). In the present study we investigated whether

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repeated transfers of P_2-reactive T cells give rise to chronic relapsing inflammatory peripheral neuritis and to what extent such a disease mimics the lesions of actively induced chronic EAN.

MATERIAL AND METHODS

T lymphocyte lines

A P_2-specific T cell line was prepared according to Linington *et al.* (1984). In short, Lewis rats were immunized in the footpad with 100 µg/animal of bovine P_2 protein in complete Freund's adjuvant. Popliteal lymph nodes were taken after 1 wk, and lymphocytes were restimulated in vitro with P_2. A cell line was developed by repeated cycles of restimulation of cultured T cells with 1 µg/ml P_2 in the presence of irradiated Lewis thymus cells as antigen-presenting cells and by expansion of the stimulated T cells with medium containing interleukin 2. After a few cycles of restimulation the cells were specific for the P_2 antigen (stimulation indices between 50 and 100) and did not react with myelin basic protein The cell lines were positive for the rat T cell markers W3/13 and W3/25 (CD4) but negative for Ox8 (CD8). Cells used to induce disease had undergone between 10 and 20 cycles of restimulation.

Passive transfer of EAN and clinical evaluation

Different cell lines have different disease-inducing capacities and even a single cell clone can change its disease-inducing capability, while still recognizing the same epitope on P_2-protein. Thus T cell numbers, which induced a reproducible mild form of EAN, were determined by repeated titration of the line used in the present experiment. 3×10^5 and 5×10^6 freshly activated P_2 T cell lines at day 3 after restimulation were given intravenously to naive Lewis rats (6–8 wks old at the first transfer) to induce the first episode of acute EAN and further relapses. The animals were weighed and scored daily using a 6 point score: 1 = tail weakness, 2 = tail paralysis, 3 = 2+hindlimb weakness, 4 = 2+hindlimb paralysis, 5 = 4+forelimb weakness, 6 = tetraplegia. Twenty-one animals were killed for pathological evaluation between 7 and 310 days. Three acute stage animals (injected with a single dose of 5×10^6 cells) were killed on day 7 after a single cell transfer, 6 animals were in chronic active stage 7 days after the last of 2–7 cell transfers, 5 animals were in a recovery stage 27 days after the last of 5 cell transfers, and 7 animals were in chronic remission between 7 and 30 wks after the last of 1–5 cell transfers. Six uninjected normal animals served as controls for quantitative immunocytochemistry.

Pathological evaluation

Animals were killed by an overdose of ether and perfused via the aorta with 4% freshly prepared paraformaldehyde, dissolved in 0.1 M phosphate-buffered saline (pH 7.4). Brain, spinal cord, cranial and spinal roots, thoracic and lumbar spinal ganglia, trigeminal ganglia and sciatic nerves were dissected. Part of the material was embedded in paraffin for light microscopy and immunocytochemistry The rest was postfixed in 3% phosphate-buffered glutaraldehyde, osmicated and embedded in Epon for electron microscopy. The following tissue samples were analysed from each animal.

Paraffin material. Six complete coronal sections of the brain, 20 complete cross-sections of the spinal cord at different levels including spinal roots and ganglia, 1 trigeminal ganglion and 1 sciatic nerve.

Epon-embedded material. Ten complete cross-sections of the spinal cord from the levels C5,6, T8,9, T13, L1 and L5,6,7 (as defined by Zeman and Maitland Innes, 1963) including the respective root entry and exit zones, 45 cross-sections of cauda equina roots at the spinal cord level L5–7 as well as 5 blocks each from sciatic nerve and trigeminal ganglion.

Paraffin sections were stained with haematoxylin/eosin, Klüver/PAS myelin stain and with Bodian silver impregnation for axons. In addition, immunostaining was performed with the following monoclonal antibodies: W3/13 (T lymphocytes and granulocytes, Seralab, GB), Ox6 (Ia antigen, Seralab, GB), ED1 (macrophages, Serotec, GB), Ox39 (interleukin-2 receptor, Serotec, GB), SMI31 (neurofilament, 200 kDa, phosphorylated determinant, Sternberger and Meyer, USA) and MCA3 (myelin basic protein, Serotec, GB). Immunocytochemistry was performed with a biotin/avidin or an alkaline phosphatase/anti-alkaline phosphatase technique as described in detail earlier (Lassmann *et al.*, 1986; Vass *et al.*, 1989).

Osmicated, Epon-embedded material was cut on a Reichert Ultracut. Light microscopic plastic sections were stained with toluidine blue. Selected areas were trimmed for electron microscopy. Sections were stained with uranyl acetate and lead citrate and examined in a Jeol S-100 electron microscope.

Quantitative evaluation and statistical analysis

Inflammation was quantified in standardized regions including 5 complete cross-sections of the lumbar and thoracic spinal cord and in consecutive longitudinal sections of the lumbar spinal roots and the sciatic nerve. T lymphocytes, Ia-positive cells and macrophages were counted in all animals in sections immunostained with W3/13, Ox39, Ox6 and ED1. The standardized area evaluated per animal was 18 mm^2 in the spinal cord and 0.4 mm^2 in the spinal roots or sciatic nerves, respectively. Since the lesions appeared to be randomly distributed throughout ventral and dorsal roots, quantification was performed on serial longitudinal sections of ventral roots L1 and L2. In the spinal cord ED1-positive macrophages were counted over the whole cross-section as well as separately in the grey matter of the anterior horn. The values given in fig. 3 are mean numbers of immunostained cells per mm^2. Statistical significance was evaluated using the Student's t test.

RESULTS

Clinical disease

A typical clinical course is depicted in fig. 1. In this experiment 5 young adult Lewis rats (180 g) were injected at day 0 with 3×10^5 of P_2-specific T cell lines. First clinical signs were observed at day 5 after cell transfer, and consisted of weight loss and ascending

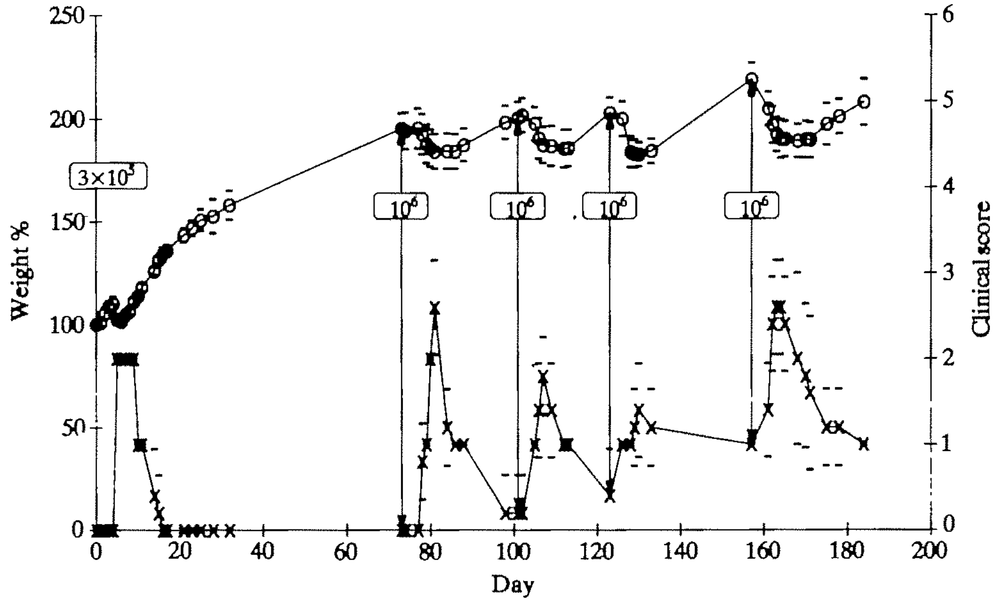

FIG 1 Clinical disease course of chronic relapsing allergic neuritis induced by repeated transfers of neuritogenic T lymphocytes A group of 5 animals was initially injected with 3×10^5 T cells; relapses were induced by intravenous injection of 10^6 T cells. Each transfer was followed by an exacerbation of the disease, reflected by increased clinical scores and weight loss After the third disease episode, recovery was incomplete. Day = days after the first transfer. Weight % = relative weight as a percentage of the initial weight at the time of the first cell transfer. Circles represent means and bars SDs. Clinical score = means (crosses) and SDs (bars) of clinical scores according to the scale described in Material and Methods

flaccid paresis. With higher cell doses ($2-5\times10^6$) first clinical signs were already observed at day 4. The maximum of clinical disease was reached around day 7 after cell transfer. Animals injected with a low cell dose, leading to mild disease (limp tail), recovered completely after 2–3 wks, whereas animals with high dose disease (hindlimb paraplegia) did not recover completely from the initial bout. Mild residual signs were still observed up to 7 mos after the initial cell transfer. Contrary to actively induced disease (Craggs *et al.*, 1986) we did not, however, observe spontaneous clinical relapses. For the induction of relapses, the animals were injected with P_2-reactive T cells (fig. 1), which was followed after 5 days by an increased flaccid paresis and weight loss. The animals again partly recovered after 2 wks or so. Multiple injections with P_2-specific T cell line cells did not lead to a reduced susceptibility to disease induction by cell transfer, i.e., we did not observe a vaccination effect by transferred T cells.

Inflammatory reaction

The inflammatory reaction in spinal roots and sciatic nerves (fig. 2) was strikingly similar, regardless of whether animals received a single or multiple transfers of neuritogenic T lymphocytes. Statistical analysis revealed no difference in the number of T cells, macrophages and Ia-positive cells 7 days after the last transfer between animals receiving 1, 2, 3 or 7 transfers of P_2-reactive lymphocytes. Similarly, no significant correlation was found between the number of inflammatory cells in the lesions and the number of transferred T cells used for disease induction. Thus in the quantitative results (fig. 3), animals are grouped according to the time after the last transfer rather than according to the total number of individual transfers or the total number of transferred cells.

During active disease (7 days after the last transfer of P_2-protein-reactive T lymphocytes) the pathological lesions were characterized by infiltration of the lesions with T lymphocytes and macrophages (fig. 2A, C, D), accompanied by a high level of Ia expression. T lymphocytes, macrophages and Ia-positive cells were located in a perivascular position as well as diffusely dispersed in the endoneurium. As described earlier in PNS lesions of allergic encephalomyelitis (Lassmann *et al.*, 1986), Ia reactivity in peripheral nerve fibres was restricted to those with acute myelin damage. Since in these fibres, ED1-positive macrophages were invariably present, it was not possible to identify, unequivocally, Ia reaction product on Schwann cells. The interleukin-2 receptor (Il2R), which is only expressed on activated lymphocytes, was visualized only on some scattered cells; most of them had entered the endoneurial space. The number of Il2R expessing cells per unit area was highly variable both in acute EAN and acute relapses (fig. 3).

During the recovery from EAN episodes (27 days after the last transfer) and during stable remissions (45–210 days after transfer) the number of T lymphocytes and Ia-positive cells in the lesions was significantly lower than in the early stages (fig. 3). In both the chronic recovery and the chronic inactive group hardly any Il2R-expressing cells were labelled with the Ox39 antibody. Single positive cells were found only within some scattered inflammatory lesions. However, a high number of macrophages, expressing ED1 antigen, persisted in the lesions.

In the CNS, perivascular inflammatory cuffs were restricted to the meninges. However, in spite of the absence of overt inflammation in the spinal cord, a significant increase

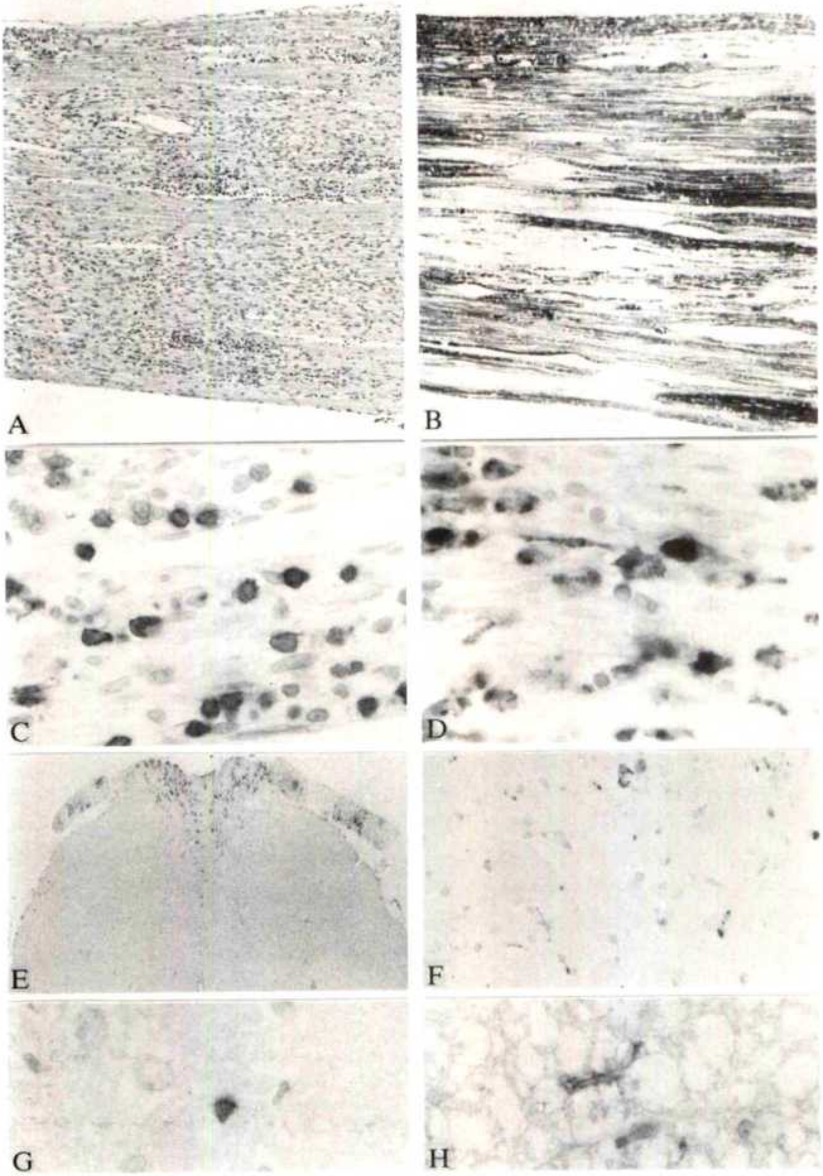

FIG. 2. Light microscopic alterations in the spinal roots and spinal cord in a rat 7 days after the seventh relapse, 340 days after the first transfer of neuritogenic T cells. A, cauda equina root with extensive inflammatory reaction; haematoxylin/eosin ×120. B, adjacent section to A showing patchy loss of myelinated fibres; Klüver-Barrera, ×120. C, adjacent section, immunostained with W3/13 (T lymphocytes); numerous T cells are present in the lesions; ×700. D, adjacent section, immunostained with ED1 (macrophages); there are numerous cells with granular intracytoplasmic reactivity in the lesions; ×700. E, lumbar spinal cord immunostained with ED1 (macrophages); there are accumulations of macrophages in the dorsal columns, reflecting areas of wallerian degeneration; ×27. F, anterior horn of the lumbar spinal cord, stained with ED1 (macrophages), showing several ED1-positive dendritic cells in the anterior horn; ×270. G, anterior horn of the lumbar spinal cord, stained with W3/13, showing a T lymphocyte in the parenchyma; ×700. H, white matter of the lumbar spinal cord stained with Ox6 (Ia antigen) showing an Ia-positive cell, probably representing an activated microglial cell; ×700.

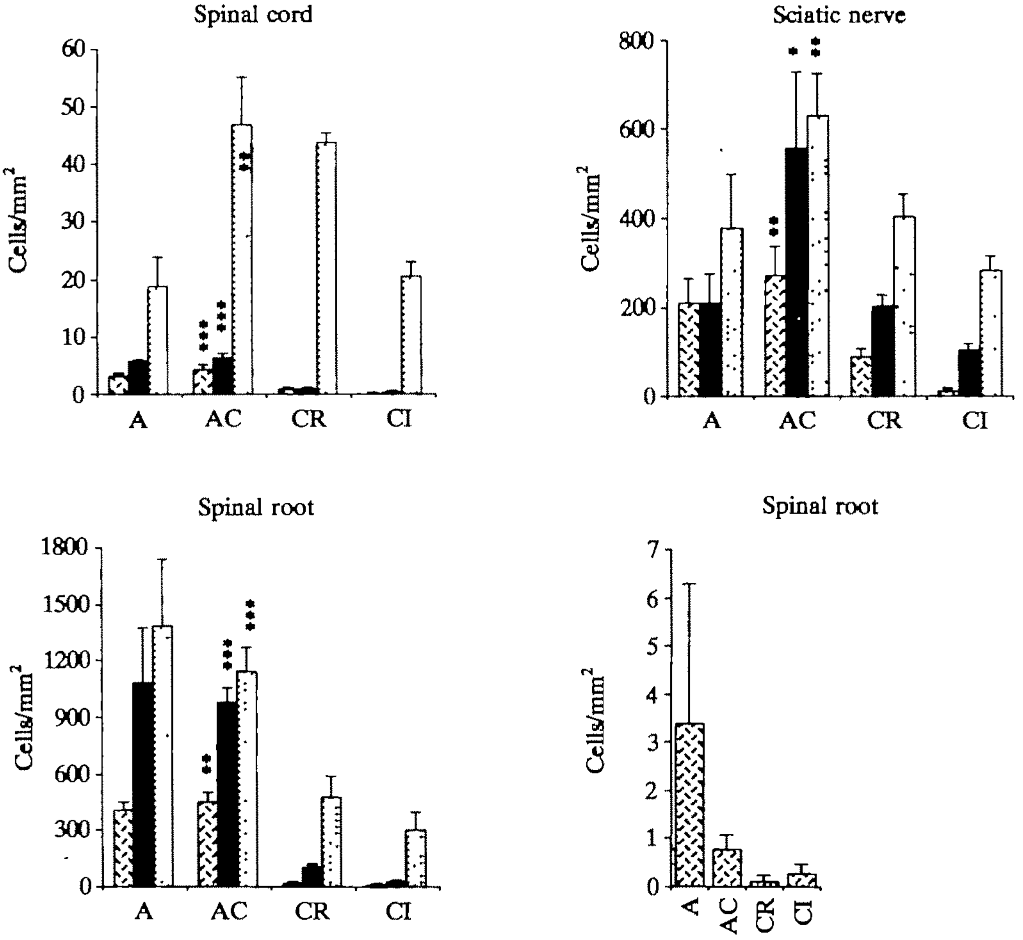

FIG. 3. Quantitative evaluation of T cells (W3/13), interleukin 2 receptor positive cells (Ox39), Ia antigen-positive cells (Ox6), and macrophages (ED1) in spinal roots, sciatic nerve and spinal cord at various stages of chronic relapsing allergic neuritis. The values represent mean values and SEMs of immunostained cells per mm^2 of tissue. A = acute EAN (7 days after the first cell transfer); AC = active chronic disease (7 days after the last transfer); CR = chronic recovery stage (27 days after the last transfer); CI = chronic inactive, stable remission phase (45–210 days after the last transfer). Groups AC and CI were compared statistically with each other using Student's t test. $*P < 0.02$, $**P < 0.01$; $***P < 0.001$. Herringbone bars = W3/13; hatched bars = Ox6, spotted bars = ED1

in the number of T lymphocytes and Ia-positive cells was found during the active stage of the disease (7 days after the last transfer; fig. 2G, H; fig. 3). These cells were diffusely dispersed in the spinal cord parenchyma, without preference for the white or grey matter. During the remission phase of the disease the number of T cells and Ia-positive cells in the spinal cord was similar to that found in normal control animals. No Il2R positive cells were found in the CNS.

During all stages of the disease ED1-positive macrophages were present in high numbers (fig. 3).

Structural lesions in the peripheral nervous system

The alterations in the peripheral nervous system were similar in all investigated areas (spinal roots, root entry and exit zones, trigeminal ganglion and sciatic nerve). During active disease inflammation of the PNS, associated with endoneurial oedema and myelin loss (fig. 2A, B), was pronounced. Nerve fibres in affected regions mainly showed changes of acute axonal degeneration with secondary myelin destruction. This was reflected by numerous axon spheroids and by the abundance of degenerated nerve fibres devoid of axons (fig. 4A, B). In addition, many nerve fibres had a widened periaxonal space (figs 4B, 5C).

Apart from these changes, a small number of nerve fibres showed signs of selective primary demyelination (fig. 5A–C). In these cases denuded axons were associated with macrophages containing myelin degeneration products; other axons had abnormally thin myelin sheaths, indicating remyelination (fig. 5D).

A quantitative estimate of the incidence of wallerian degeneration and primary demyelination in 3 animals in the acute stage of chronic EAN showed that 85% of pathological fibres revealed primary axonal damage. In contrast, only 15% showed signs of primary demyelination.

During remissions (more than 45 days after the last transfer) changes in peripheral nerves and roots were mainly reflected by a shift of the myelinated fibre spectrum towards fibres with small diameters (fig. 6A). These fibres were grouped in clusters (fig. 6B), sometimes surrounded by a common basement membrane. However, in addition to these changes, some large axons were surrounded by thin myelin sheaths and Schwann cell processes, resembling the pattern of primary demyelination and remyelination. No onion bulbs were found in spinal roots, trigeminal ganglia and peripheral nerves or from paraffin sections, in spinal ganglia, even in animals with 7 transfers of P_2-reactive T cells.

Structural damage in the central nervous system

Throughout all stages of the disease increased numbers of macrophages were present in the dorsal columns of the spinal cord (fig. 2E). These alterations were accompanied by a variable degree of axonal loss, myelin degeneration and reactive gliosis.

In addition, increased numbers of ED1-positive macrophages were also present in the anterior and posterior horns of the cord (fig. 2F). In the anterior horn ED1-positive cells are rare in control animals (2.3 ± 2.0 cells/mm^2) and are located exclusively in a perivascular position. In EAN, ED1-positive cells were also found in a perineuronal location (fig. 2F). The most pronounced increase was observed during active chronic disease (110 ± 45 cells/mm^2), but elevated counts (32 ± 13 cells/mm^2) were noted even during stable remission. Nerve cells in these regions showed signs of central chromatolysis, accompanied by accumulation of phosphorylated neurofilament epitopes in the perikaryon.

DISCUSSION

The pathogenetic mechanisms involved in inflammatory demyelinating diseases of the human PNS are still not clear. Although the close similarity of the pathology between acute Guillain-Barré syndrome and acute T cell-mediated allergic neuritis (Izumo *et al.*,

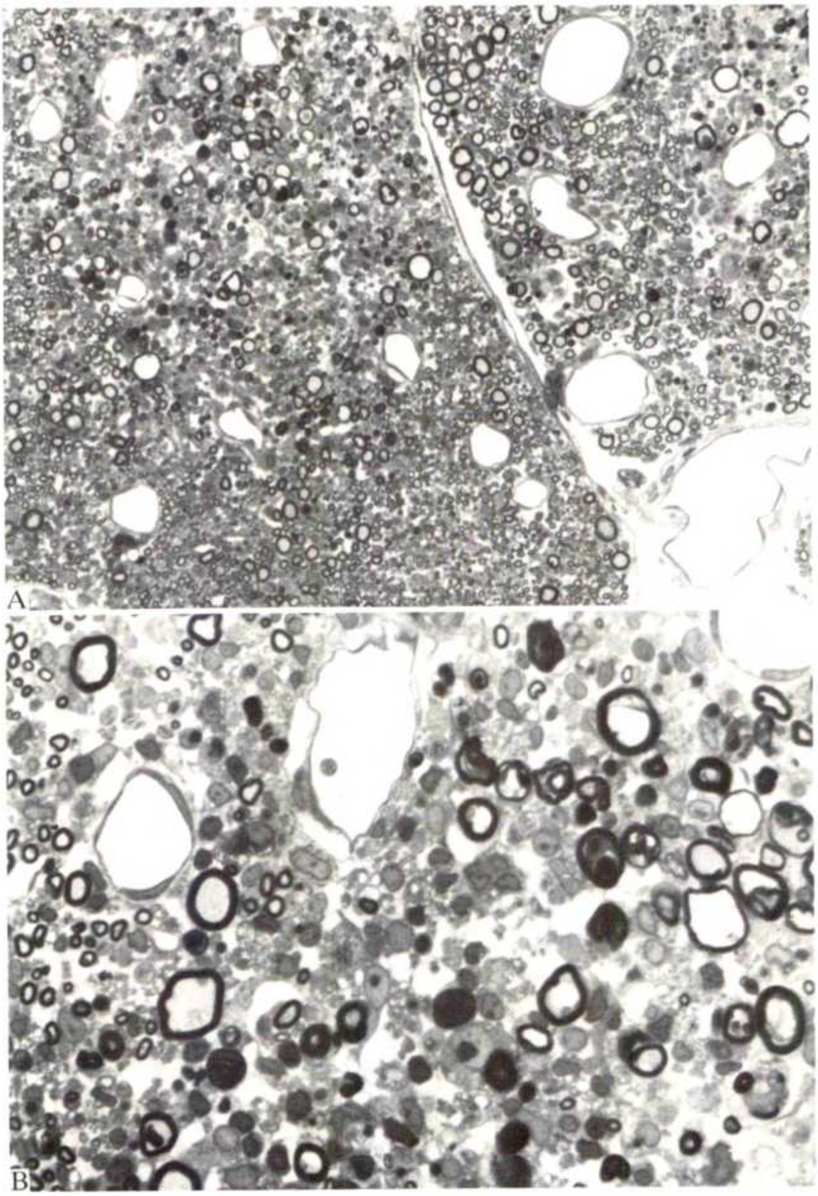

FIG. 4. Neuropathological alterations in spinal roots in a rat 7 days after the seventh relapse, 340 days after the first transfer of neuritogenic T cells. A, low power micrograph of cauda equina roots with perivascular loss of myelinated fibres and perivascular accumulation of abnormal small myelinated fibres in other areas; toluidine blue, ×330. B, detail from A showing infiltration of the endoneurium with inflammatory cells, pronounced loss of myelinated nerve fibres, absence of fibres with primary demyelination and some large fibres with swelling of the periaxonal space; toluidine blue, ×1000.

1985) suggests the operation of similar pathological mechanisms, the situation in chronic idiopathic demyelinating polyradiculoneuropathy (CIDP) may be different. In this disease, which is associated with more widespread demyelination (Prineas and McLeod, 1976;

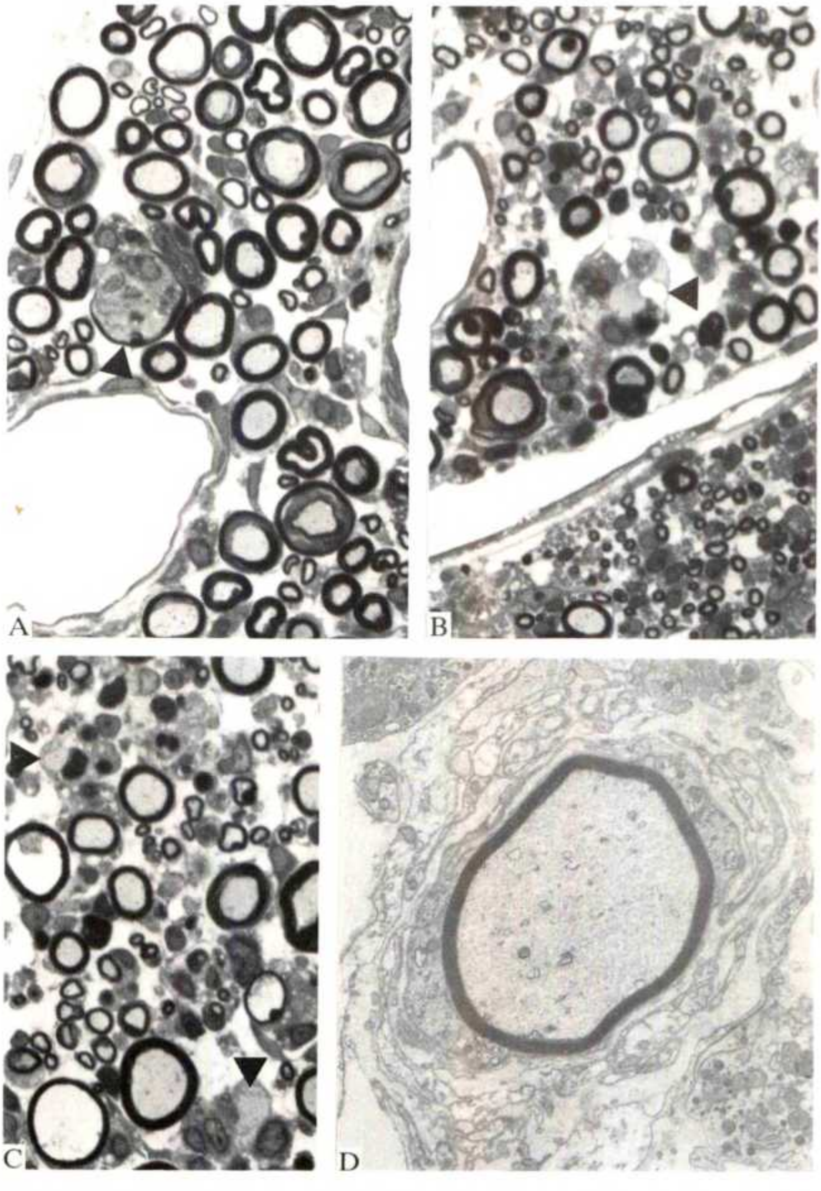

FIG. 5. Cauda equina roots from the same animal as in fig. 4 showing demyelination and remyelination. A, invasion of macrophages into a myelinated nerve fibre (arrow); toluidine blue, ×1000. B, demyelinated axon (arrow) surrounded by macrophages containing debris; toluidine blue, ×1000. C, demyelinated axons (arrows) and other nerve fibres with a widened periaxonal space; toluidine blue, ×1000. D, electron micrograph of the same spinal root showing a remyelinated nerve fibre surrounded by clusters of Schwann cell processes; ×5300.

Hughes, 1985), humoral factors such as antibodies and complement may be relevant pathogenetically (Dalakas and Engel, 1980; Toyka and Heininger, 1987). We have thus tried to define experimentally the pathological lesions of a purely T lymphocyte-mediated chronic autoimmune disease of the PNS.

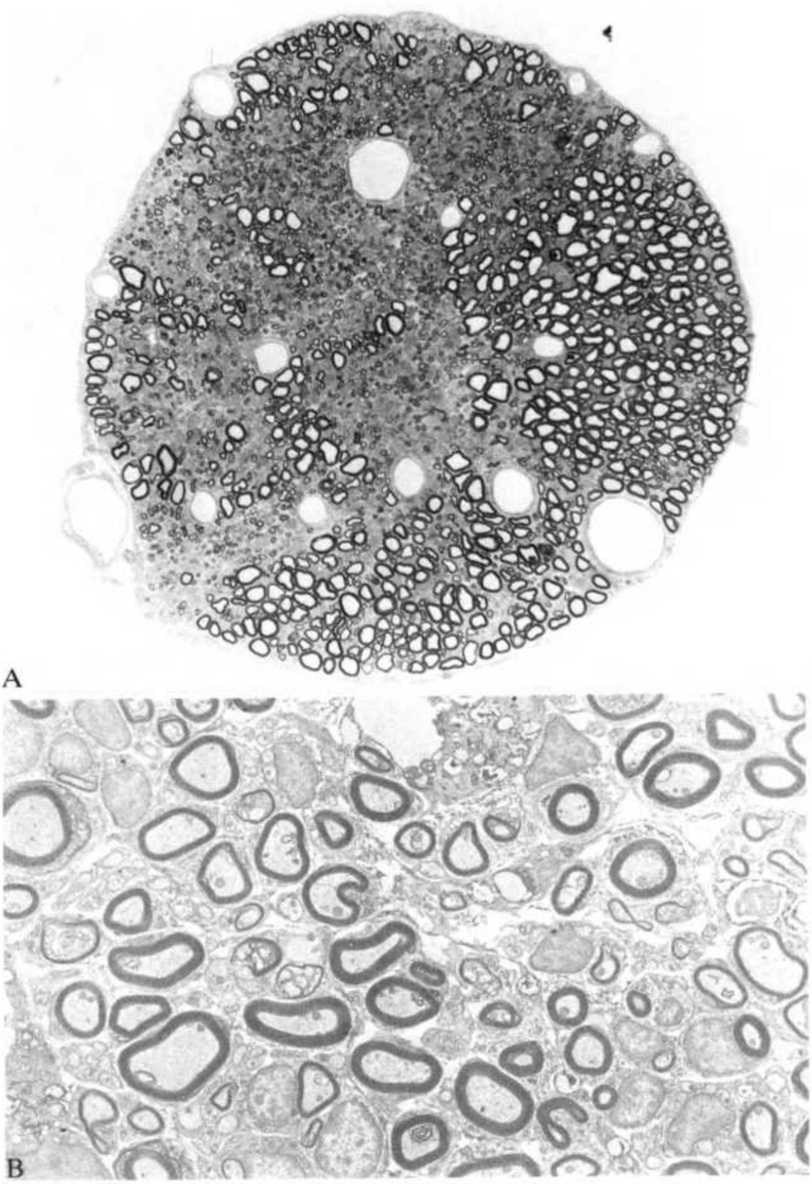

FIG. 6. Neuropathological alterations in spinal roots in a rat 137 days after the fifth relapse, 340 days after the first transfer. A, cauda equina root with a focal perivascular accumulation of abnormally thin, clustered myelinated nerve fibres; toluidine blue, ×370. B, low power electron micrograph of an area with abnormally thin myelinated fibres; the fibres are grouped in clusters; there are 2 endoneurial macrophages with lipid debris; ×2600.

Our present study revealed several results which may be relevant for the understanding of chronic peripheral neuritis. (1) Multiple transfers of monospecific T lymphocytes, directed against P_2-protein can imitate a relapsing peripheral neuritis. (2) Each transfer resulted in an immunopathological reaction in the PNS, indistinguishable from that in acute T cell-mediated EAN. (3) In chronic relapses of the disease the pathology of T cell-mediated EAN was dominated by inflammation and secondary nerve damage. Primary demyelination and remyelination was rare and onion bulbs were absent. (4) Chronic T cell-mediated EAN was associated with profound changes in the CNS, reflecting the axonal damage by secondary tract degeneration in the dorsal columns and retrograde degeneration of motor neurons. (5) Active, PNS-specific T cell-mediated disease was associated with a significant increase in the number of T lymphocytes and Ia-positive cells in the CNS.

A relapsing disease course has been described previously, induced by multiple transfers of myelin basic protein (MBP) reactive T lymphocyte lines (Vandenbark *et al.*, 1986). On the other hand, animals can be vaccinated by intravenous injection of encephalitogenic T lymphocyte lines (Ben-Nun *et al.*, 1981; Cohen and Weiner, 1988). Such animals are then refractory towards active sensitization with MBP or passive transfer of MBP reactive T cell clones. A similar vaccination effect against active induction of EAN has been observed after pretreatment of animals with a T cell line specific for P_2 protein (Taylor and Hughes, 1988). Furthermore, during the recovery stage of T cell line mediated EAE, CD8-positive T lymphocytes can be cloned from the CNS, which suppress EAE and are cytotoxic for MBP reactive CD4-positive, encephalitogenic T lymphocytes (Sun *et al.*, 1988). In our present experiment no vaccination effects were observed, in spite of the fact that for all transfers the same T cell line was used. Thus the vaccination effect may only involve subpopulations of encephalitogenic or neuritogenic T cells. Alternatively, a possible vaccination effect may have been overruled in our present experiments by the numbers of cells injected in the course of the transfers or by the long intervals between individual transfers.

Selective primary demyelination is a consistent and characteristic feature of the pathology of EAN (Waksman and Adams, 1955). Demyelination following neuritogenic T cell transfer may either be caused by direct T cell-mediated cytotoxicity or indirectly by a bystander mechanism (Hartung *et al.*, 1988), by PNS-specific demyelinating antibodies (Saida *et al.*, 1987) or by a cooperation of cellular and humoral immune mechanisms (Lassmann *et al.*, 1986, 1988; Linington *et al.*, 1988). Since Schwann cells can act facultatively as antigen-presenting cells (Wekerle *et al.*, 1986*b*) and in this process can be lysed by the antigen recognizing neuritogenic T cell lines (K. Nomura, unpublished), similar to the T cell interaction with astrocytes (Sun and Wekerle, 1986), demyelination in T cell-mediated EAN may be due to direct lymphocyte cytotoxicity. In fact, acute EAN induced by P_2-protein reactive T cells is accompanied by selective primary demyelination (Izumo *et al.*, 1985; Heininger *et al.*, 1986; Wiethölter *et al.*, 1988). Although some demyelination was also noted in the present experiments after multiple transfers of neuritogenic T cells, selective primary demyelination involved only a minority of peripheral nerve fibres and typical features of repeated demyelination and remyelination such as onion-bulb formation were absent.

Titration studies with P_2-reactive T cell lines have shown that after transfer of low T cell numbers, demyelination is selective, whereas after transfer of high numbers of T cells, secondary wallerian degeneration dominates (Izumo *et al.*, 1985; Heininger *et al.*, 1986; Hartung *et al.*, 1988). Thus the low amount of demyelination observed in the present study may be due to the relatively high numbers of cells used. However, the disease-inducing capacity of different cell lines differs, the line used in our present experiments being a factor of 10 less efficient compared with that used by Izumo *et al.* (1985) and Heininger *et al.* (1986). Thus we tried to find with our present T cell line a dose which induced mild clinical disease, similar to that described earlier to result in selective demyelination. This experimental procedure, however, did not lead to extensive primary demyelination in the chronic experiments. It thus remains unresolved as to whether different P_2-reactive T cell lines have different capabilities to induce selective demyelination. Alternatively, widespread demyelination and remyelination, a typical feature of actively induced chronic EAN (Wiśniewski *et al.*, 1974; Harvey *et al.*, 1987) may require additional immunological mechanisms. Recently, we were able to show that demyelination in T cell line mediated EAE can be massively augmented by circulating demyelinating antibodies (Lassmann *et al.*, 1988; Linington *et al.*, 1988). Similarly, high titres of demyelinating antibodies have been found in a model of chronic inflammatory neuritis which is associated with extensive demyelination (Lassmann *et al.*, 1986).

Repeated transfer of P_2-protein reactive neuritogenic T lymphocytes also had pronounced effects in the CNS. These effects can be classified into two groups: the consequences of axonal degeneration in the spinal roots and peripheral nerves and the increase of T cells and Ia-positive cells in the spinal cord parenchyma during the active stage of the disease.

Walleran degeneration in the peripheral nerves was reflected by tract degeneration in the dorsal columns of the cord. In addition, some retrograde reaction was found in nerve cells of the anterior horns, together with increased numbers of perineuronal macrophages. This reaction pattern is similar to that described in facial nuclei after nerve transsection (Graeber *et al.*, 1988; Streit *et al.*, 1988).

In addition, during active disease episodes of EAN we found increased numbers of T lymphocytes and Ia-positive cells in the spinal cord parenchyma. Since P_2-protein is present in bovine, rabbit and human, but absent in rat and mouse central myelin (Trapp *et al.*, 1983), this observation can best be explained by earlier studies by Wekerle *et al.* (1986*a*), which suggest that activated T lymphocytes may reach the CNS in an antigen-unspecific way. In these experiments few labelled cells were found in the CNS after intravenous injection of ovalbumin reactive T lymphocytes, indicating that migration of T lymphocytes to the CNS depends on their state of activation rather than on an antigen-specific recognition event. Thus such cells may represent the initial step of immune surveillance of the CNS in the course of infections or autoimmune disease (Wekerle *et al.*, 1986*a*).

In summary, our present experiments show that chronic relapsing inflammatory neuritis can be induced by multiple transfers of P_2-protein reactive T lymphocytes. The highly standardized clinical course and pathological reaction commends this model for studies on the pathogenesis and therapy of the lesions. The pathology in this model was dominated by inflammation but, contrary to actively induced EAN, demyelination was sparse.

ACKNOWLEDGEMENTS

The study was funded by the Science Research Fund, Austria, Project No. P 6438M. W.F. was supported by the Cloetta Foundation and the Swiss National Science Foundation (Project No. 3.998.0.86). We thank I. Peterhans, H. Breitschopf, A. Cervenka, S. Katzensteiner and M. Leisser for excellent technical assistance.

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(*Received September 20, 1989. Revised December 29, 1989. Accepted March 15, 1990*)

Brain (1991), 114, 443–460

MODULATION OF THE STRETCH REFLEX DURING VOLITIONAL SINUSOIDAL TRACKING IN PARKINSON'S DISEASE

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SUMMARY

Sinusoidal visually-guided wrist tracking, in normal and parkinsonian subjects, was perturbed by torque transients every 90° throughout the movement. Long-latency stretch reflex and volitional EMG amplitude modulations were assessed as functions of the tracking phase. Reflex modulation during tracking, both in wrist flexor and extensor muscles, was found to differ significantly between parkinsonian and normal subjects. In the parkinsonian group, the abnormality consisted of an increased reflex activity during tracking phases in which the muscle was lengthening. At these phases the reflex generated torque is opposite in direction to the volitionally generated torque and the tracking movement. No differences in the unperturbed volitional EMG modulation were observed between groups for this error constrained tracking paradigm. Significant correlations were found between ratings of bradykinesia and the amount of abnormal reflex modulation in the wrist flexor. These data suggest that a component of bradykinesia results from a defective coordination of supraspinal reflex and volitional control systems.

INTRODUCTION

Significant modulation of the long-latency stretch evoked responses occurs during volitional movements in normal subjects (Gottlieb and Agarwal, 1973, 1978; Dufresne *et al.*, 1980; Soechting *et al.*, 1981; MacKay *et al.*, 1983). In cyclical movements of the elbow, the amplitude of the stretch reflex increases during or just preceding the volitional muscle shortening and markedly decreases during lengthening (Dufresne *et al.*, 1980; MacKay *et al.*, 1983). This comodulation of reflex and volitional EMG activity generally conforms to predictions based on the postulate of servo-assistance (Matthews, 1964; Stein, 1974) and is consistent with the concept of an 'automatic gain control' servo action during volitional contraction (Marsden *et al.*, 1972, 1976).

Multiple reports of increased long-latency reflex amplitude in parkinsonism have been documented utilizing perturbations applied to a statically positioned skeletal joint (Tatton and Lee, 1975; Marsden *et al.*, 1978; Berardelli *et al.*, 1983; Rothwell *et al.*, 1983; Cody *et al.*, 1986). The present study examines the modulation of the long-latency stretch reflex during volitional sinusoidal movement of the wrist in parkinsonian subjects. It was hypothesized that analogous changes in the reflex amplitude modulation may be present during volitional tracking. When compared with normals, modulation of the

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stretch reflex during sinusoidal movement was found to be altered significantly in parkinsonism. Elevated reflex activity occurred during phases of movement in which the muscle was lengthening. A consequence of this substantial reflex activity in those phases of movement during which the muscle is stretched is the impediment of volitional movement. A preliminary report of this work has been presented in abstract form (Johnson *et al.*, 1989).

METHODS

Subjects

The parkinsonian subject group consisted of 15 adults (7 males, 8 females) aged 47 – 81 (mean 64.6) yrs. These subjects were studied during routine clinic appointments. All but 1 were on medication (Table 1), with the last dose occurring the night before the testing. Parkinsonian subjects were selected on the basis of absence of other neuromuscular or skeletal disease, and ability to track volitionally a ±36°, 0.25 Hz sinusoid with the wrist. The dominant extremity was tested in all subjects. The age-matched normal group consisted of 15 neurologically normal adults (10 males, 5 females), aged 51 – 68 (mean 56.3) yrs. Normal subjects had no history or evidence of skeletal or muscular disturbances such as arthritis or trauma. All subjects were studied after informed consent was obtained.

TABLE 1 CLINICAL PROFILE OF PARKINSONIAN GROUP

Case	*Age (yrs)*	*Sex*	*Diagnosis*[1]	*Disease duration (yrs)*	*Medication*[2]	*Bradykinesia*[3]	*Rigidity*[3]	*Stage*[4]
1	58	M	IP	7	LD7	1	2	II
2	64	F	IP	10	LD7, B2	3	2	III
3	47	F	CVP	2	LD2	1	2	I
4	73	M	IP	22	LD16	3	2	III
5	66	M	IP	24	LD17; B10	2	2	III
6	73	M	IP	8	LD5	2	2	III
7	65	F	IP	4	LD2	1	1	I
8	72	F	IP	3	LD1	1	2	I
9	53	F	IP	5	LD2	0	2	I
10	72	F	IP	3	LD1	0	2	I
11	54	F	IP	3	LD1	0	1	I
12	59	M	IP	4	LD3	0	1	I
13	67	F	IP	5	LD3	0	2	II
14	81	M	IP	23	LD18	4	3	IV
15	65	M	IP	2	–	0	1	I

[1] IP = idiopathic parkinsonism; CVP = parkinsonism secondary to ischaemic cerebrovascular disease; [2] LD = levodopa, B = bromocriptine, numbers = duration of treatment in yrs; [3] 0 – 4, New York University Scale, [4] I – IV, Hoehn and Yahr scale.

Apparatus and data acquisition

The experiment involved tracking a sinusoidally driven target with the wrist. During volitional tracking with acceptable error, flexion and extension directed torque perturbations were randomly introduced at the 0°, 90° (maximum extension), 180°, and 270° (maximum flexion) phases of the tracking cycle. Stretch-evoked EMG and volitional EMG were acquired at these phases.

The subject was seated with the forearm horizontally immobilized with the ulnar axis rotationally fixed, neutral with respect to pronation-supination, in such a way as not to impede the sliding of tendons or the flexion-extension movement of the wrist joint. The hand of the subject was secured to a low mass handle designed to keep the hand in a position of functional grasp. Neutral position, defined as the mean angular position of the tracking sinusoid, was chosen to reflect the anatomically relaxed wrist position between flexion and extension. The d.c. torque motor (Electrocraft, Gallipolis, Ohio, model 644-06-011, torque constant = 0.307 N·m/A, driven by a high impedance current source amplifier) and precision potentiometer were orientated along a vertical axis in line with the axis of rotation of the wrist joint. A closed-loop microprocessor-based control scheme was used to display a sinusoidal tracking target, deliver torque step transients to the wrist at specified tracking phases, and acquire position and EMG data. Wrist position was displayed to the subject by a horizontal bar display, consisting of a 10 cm long linear array of 101 light-emitting diodes (LEDs). Similarly, a computer-generated target bar incorporating a second array of LEDs was displayed above the wrist position bar. The target display was driven by a 0.25 Hz sinusoid. Amplitude of the torque step was 0.5 or 0.75 N·m (Newton·metre) with a duration of 250 ms. The direction (flexion or extension) of the transient applied to the wrist was randomized. At a randomly selected phase along the tracking sinusoid (specifically 0°, 90° (maximum extension), 180°, 270° (maximum flexion)), either a flexion torque transient, an extension torque transient, or no torque transient was randomly delivered. No further perturbations followed until a full intervening tracking cycle was completed. The torque transient was delivered only when the subject's position matched the computer-generated tracking signal within an error margin specified as a function of movement amplitude ($\pm 3°$) and movement phase interval ($\pm$ 100 ms) from a potential perturbation delivery site. This ensured stereotyped sinusoidal tracking at the time of perturbation.

The EMG signal was differentially recorded from circular Ag-AgCl electrodes, 4 mm in diameter, placed 6–10 cm apart over the muscle bellies of the extensor carpi radialis and flexor carpi ulnaris. The reference electrode was placed between the sensing electrodes. The EMG signal was amplified (gain of 5000 to 20 000), and band-pass filtered with 3 dB cut-off frequencies of 10 and 300 Hz. The angular position of the wrist and EMG of the extensor and flexor musculature were sampled at 2000 samples/s, and digitized with 12 bit resolution. The acquired EMG signal was nulled to amplifier offset voltage and rectified digitally. Each perturbation initiated a sample data record of 200 ms duration for reflex EMG activity and 400 ms duration for position. As a measure of volitional activity, flexor and extensor EMG records of 200 ms duration were acquired at each trigger phase when no perturbation was delivered. The term, volitional EMG, will refer to this unperturbed EMG activity. The unperturbed EMG baseline was subtracted from the corresponding stretch-evoked EMG at each perturbation phase to yield a measure referred to as the reflex EMG (after Dufresne *et al.*, 1980; MacKay *et al.*, 1983). Subtraction and division by the baseline were both explored and found to give similar results. The sampled points were averaged into bins of 10 ms for EMG and 20 ms for position. Averaging was performed within each bin and over the number of repetitions of each perturbation category (i.e., tracking phase and perturbation direction). After a preliminary series of tests, only binned values of position and EMG were recorded due to storage limitations. Fig 2 demonstrates the effect of binning on the morphology of stretch-evoked EMG.

Procedure

Subjects were instructed to match the two displayed bars by alternately flexing and extending the wrist to follow the computer-generated tracking signal. The subjects were instructed to track in a relaxed fashion and were not to react to the torque pulses. Instructions to the subject discouraged active grasping of the handle, as this may have activated the long finger muscles and contaminated the wrist flexor and extensor EMG. A practice session, consisting of sinusoidal tracking of a sufficient duration, so that 5 to 10 repetitions of all perturbations occurred, was initially performed by all subjects. Two measurement series of 10 repetitions for each movement phase perturbation were performed. As no distinct sequential trends in the data were ascertained, these two series were subsequently averaged. Normal subjects could perform each series in approximately 12 min. Severely symptomatic parkinsonian subjects often required more than 1 h, as their performance often failed the tracking error criterion and transients were delivered less frequently. Subjects were encouraged to take frequent rest periods.

RESULTS

Volitional EMG, reflex EMG, and position were measured only when the tracking performance fell within a specified error. This constrained the tracking amplitude and phase, resulting in roughly similar volitional EMG and tracked movement for normals and parkinsonians. Averaged volitional EMG from the flexor and extensor, and tracking movement are shown for a normal subject (A–C) and for a parkinsonian subject (D–F) during unperturbed sinusoidal tracking in fig. 1. The amount of coactivation and the depth of modulation of the volitional EMG in these two cases are similar (*see also* Table 2).

In fig. 2, the flexor muscle reflex EMG and associated extension deflection produced by an extension-directed torque pulse of 0.5 N·m are shown for the normal and parkinsonian subject of fig. 1. The perturbation occurred at 0° phase, during the ±36°

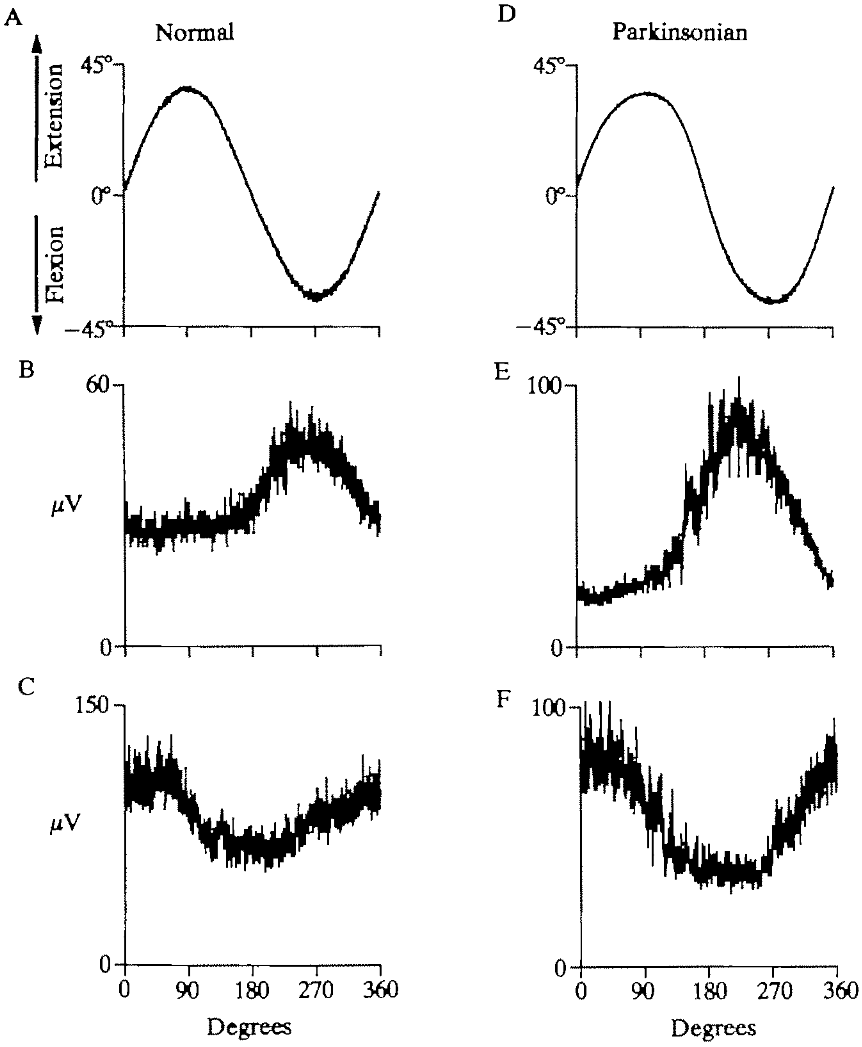

FIG. 1 Volitional EMG and tracked movement traces for normal (A–C) and parkinsonian (D–F) subjects. Unperturbed ±36° sinusoidal tracking, average of 50 repetitions Horizontal axis is tracking phase in degrees A and D are wrist displacement from the neutral point (in degrees, with positive values corresponding to extensions). B and E are the associated flexor muscle EMG in μV. C and F are the associated extensor EMG in μV

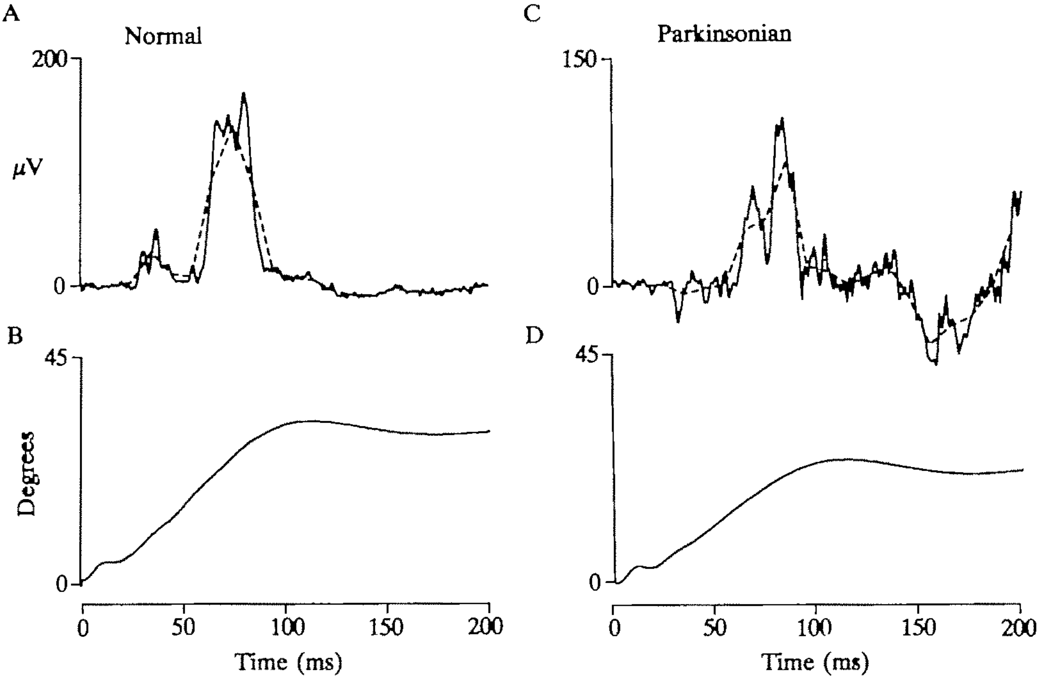

FIG. 2 Reflex EMG of the flexor and associated deflection, evoked by an extension directed torque perturbation at 0° phase during sinusoidal tracking A and C are the reflex EMG responses for a normal and parkinsonian subject. Unbinned (2000 samples/s. solid lines) and binned (10 ms bins: broken lines) EMG responses are compared. B and D are the wrist deflections into extension. Traces are averages of 20 repetitions. Horizontal axis is time in ms from onset of torque perturbation.

tracking cycle. The resultant extension deflection and flexor muscle reflex EMG are shown for a normal (A, B) and a parkinsonian subject (C, D). In both subjects, the evoked EMG responses are morphologically similar to previous descriptions (Tatton and Lee, 1975; Rothwell *et al.*, 1983). The amplitude and time course of the deflection is somewhat smaller and slower for this parkinsonian subject (B vs D). Group comparisons of compliance modulation as a function of tracking phase were made. Measurements of wrist deflection at 50, 100, and 200 ms after perturbation, at 0° and 180° tracking phases demonstrated no statistically significant compliance modulation differences between the normal and parkinsonian groups. This parallels the finding of no difference in compliance between normal and parkinsonian groups when measured by perturbation of a statically positioned joint (Rothwell *et al.*, 1983).

The extensor muscle reflex EMG evoked in 90° phase intervals for a normal and a parkinsonian subject is shown in fig. 3. The long-latency reflex activity (40–120 ms postperturbation) predominates. Reflex modulation in the normal group is comparable with results obtained from torque perturbations of volitional elbow tracking (Dufresne *et al.*, 1980; MacKay *et al.*, 1983). For the normal, the stretch reflex in the wrist extensor is maximal at 0° tracking phase, when the extensor is actively contracting and shortening (*see* fig. 1). Conversely, reflex amplitude decreases at 180° when the wrist is flexing and the mucle lengthening. Peak reflex activity modulates by a factor of 8 with tracking phase in this case. Although the parkinsonian reflex responses exhibit a similar degree

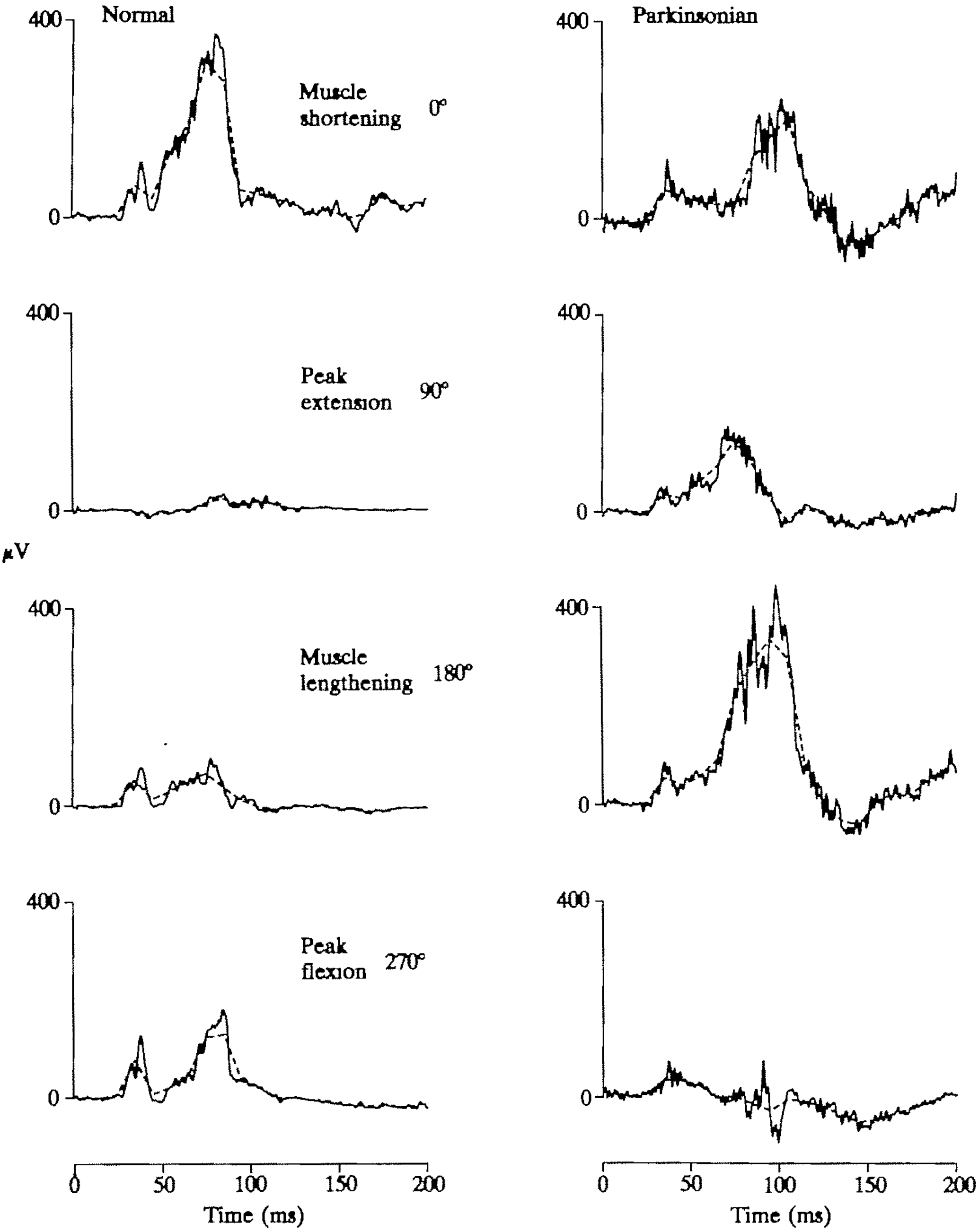

Fig. 3. Reflex EMG of the wrist extensor assayed at 0°, 90° (maximum extension), 180°, and 270° (maximum flexion) for a normal subject (*left column*) and parkinsonian subject (*right column*). The horizontal axis is time from perturbation in ms. The vertical axis is amplitude in μV. All traces are averages of 20 repetitions. Solid traces are unbinned and broken lines binned responses as described in fig. 2.

of modulation with the phase of tracking, the modulation is considerably different from that of the normal. Maximal extensor reflex amplitude occurs at 180° and a large component exists at 90°, phases which typically exhibit minimal extensor reflex amplitude in normals.

Fig. 4 presents the profiles of reflex EMG and volitional EMG as functions of tracking phase for the same subjects as in fig. 3. The reflex EMG of the wrist extensor for the normal and parkinsonian subjects is shown in A. The normal subject demonstrates maximum reflex EMG at 0° as the extensor is shortening volitionally. For the parkinsonian subject, the peak extensor reflex EMG occurred at 180°, as the extensor is lengthening. The unperturbed extensor volitional EMG for the subjects is shown in C. Modulations are similar with the amplitude increasing during phases of tracking into extension. The reflex EMG from the flexor muscle for the normal and parkinsonian subjects is shown in B. Both subjects demonstrated similar reflex modulation profiles with maxima at 180°, when the wrist flexor is contracting volitionally. The illustrated parkinsonian subject falls into the sample subgroup later described as having primarily extensor reflex changes. Unperturbed volitional EMG from the wrist flexor is similar in both subjects, with the envelope increasing as the subject tracks into flexion (D).

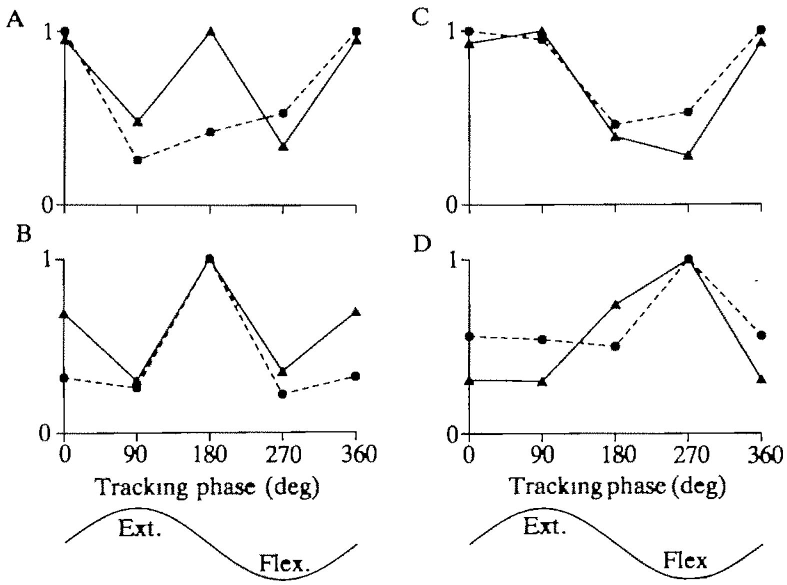

FIG 4. Reflex EMG (*left*) and volitional EMG modulation (*right*) for the same normal (circular symbols, broken lines) and parkinsonian subject (triangular symbols, solid lines) as in fig. 3. The EMG activity, summed over the postperturbation interval from 40–120 ms, is plotted as a function of tracking phase (horizontal axis). A schematic of the tracking movement noting flexion and extension directions is depicted beneath the phase axis. Amplitudes are normalized to peak value to show modulation. A, extensor muscle reflex EMG modulation. B, flexor muscle reflex EMG modulation. C, extensor volitional EMG modulation D, flexor volitional EMG modulation.

In order to visualize the modulation of the reflex activity, contour plots of the reflex amplitude as a function of time following perturbation and tracking phase were constructed. Examples of the resultant contour plots for normal and parkinsonian subjects are presented in fig. 5. The phase-time contour plots of flexor and extensor reflex EMG are shown for a selected young (age 28 yrs) normal subject in A and an age-matched normal subject in B. As present in B, increased latency of reflex activity was common in the older age-matched group. However, modulation of the reflex activity with tracking phase is similar for all normals, with distinct increases during homonymous muscle shortening. Accordingly, maximal reflex amplitude in the flexor is centred around 180° and for the extensor, the maximal amplitude is centred around 0°. At tracking phases resulting in homonymous muscle lengthening, the reflex amplitude decreases to baseline. Contour plots from 3 parkinsonian subjects are depicted in C–E. Changes in the reflex modulation, characterized by increased reflex amplitude at tracking phases in which the homonymous muscle is lengthening, are apparent in all 3 parkinsonian subjects when compared with the normals. For example in C, at 90° considerable reflex activity is evoked in the flexor as opposed to normals which exhibit a reflex peak centred at 180°, the phase at which the flexor is shortening. In D the subject demonstrates analogous changes in the modulation of the extensor reflex with increases at 180°, the phase in which the extensor is lengthening. The subject illustrated in E exhibits both flexor and extensor reflex modulation changes, again with increased reflex amplitude during homonymous muscle lengthening. Similar changes in reflex modulation are demonstrable in one or both muscles for a majority of parkinsonian subjects tested.

Indices were developed to assess the modulation of reflex amplitude with tracking phase and to compare quantitatively the parkinsonian and normal groups. The reflex log shortening/lengthening (reflex S/L) ratio is defined as the decimal logarithm of the ratio of mean reflex EMG activity occurring from 40 to 120 ms following perturbation, evoked at two tracking phases. The numerator of the ratio is the reflex activity at the phase corresponding to the maximal tracking velocity in the direction of homonymous muscle shortening. The denominator is the reflex EMG activity at the phase corresponding to the maximal tracking velocity in the direction of homonymous muscle lengthening. The logarithm of the ratio was used to linearize the scale. In all index calculations, the EMG matrix was normalized by the mean of all matrix elements to minimize differences in electrode impedance and sampling of motor unit activity. Selection of the time interval used in the index reflects the observation that the modulation of the reflex during tracking occurs primarily at longer latencies (Dufresne *et al.*, 1980). The 120 ms cut-off point was selected to minimize the inclusion of activity due to volitional reaction (Jaeger *et al.*, 1982). Selection of specific tracking phases was based on previous studies of reflex modulation in normals. It has been demonstrated that for pursuit tracking under no external load, the reflex amplitude peak is centred at the phase corresponding to the maximal tracking velocity in the direction of homonymous muscle shortening (Dufresne *et al.*, 1980; MacKay *et al.*, 1983). Also, the minimum of reflex amplitude occurs in the phases corresponding to maximal tracking velocity in the direction of muscle lengthening. In the specific example of the flexor, the maximum reflex amplitude occurs near 180° and the minimum near 0°.

Comparison of the extensor muscle reflex S/L ratio for the normal and parkinsonian groups is presented in fig. 6. A graphical description of the tracking phase and time

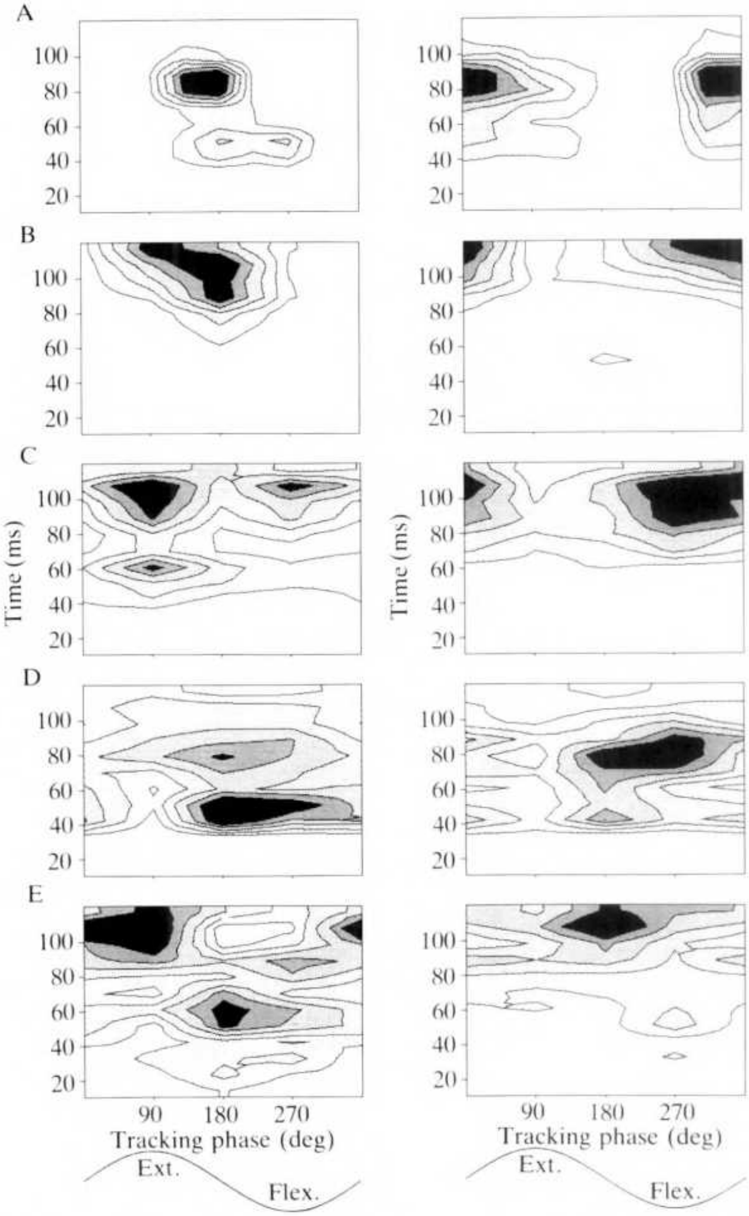

FIG. 5. Flexor (*left column*) and extensor (*right column*) reflex amplitude contours obtained from 0.25 Hz ± 36° sinusoidal tracking task perturbed by torque steps at 0°, 90° (maximum extension), 180°, 270° (maximum flexion) tracking phases. Contours were generated using the averaged EMG array consisting of 4 columns (phase of sinusoidal tracking) and 12 rows (time relative to onset of perturbation, 10 ms bins). A new grid of 12 × 36 elements was generated using inverse distance-weighted linear interpolation, and contours were drawn through interpolated isovalue points. Vertical axis is time in ms after torque transient. Horizontal axis is tracking phase in degrees. Relative amplitude of EMG is represented by grey scale. All are averages of 20 repetitions. Tracking schematic as in fig. 4. A, characteristic young normal subject (age 28 yrs). B, representative age-matched normal (age 52 yrs). C, parkinsonian subject demonstrating predominant flexor muscle reflex modulation defect. D, parkinsonian subject with predominant extensor reflex modulation defect. E, parkinsonian subject demonstrating both flexor and extensor modulation defects.

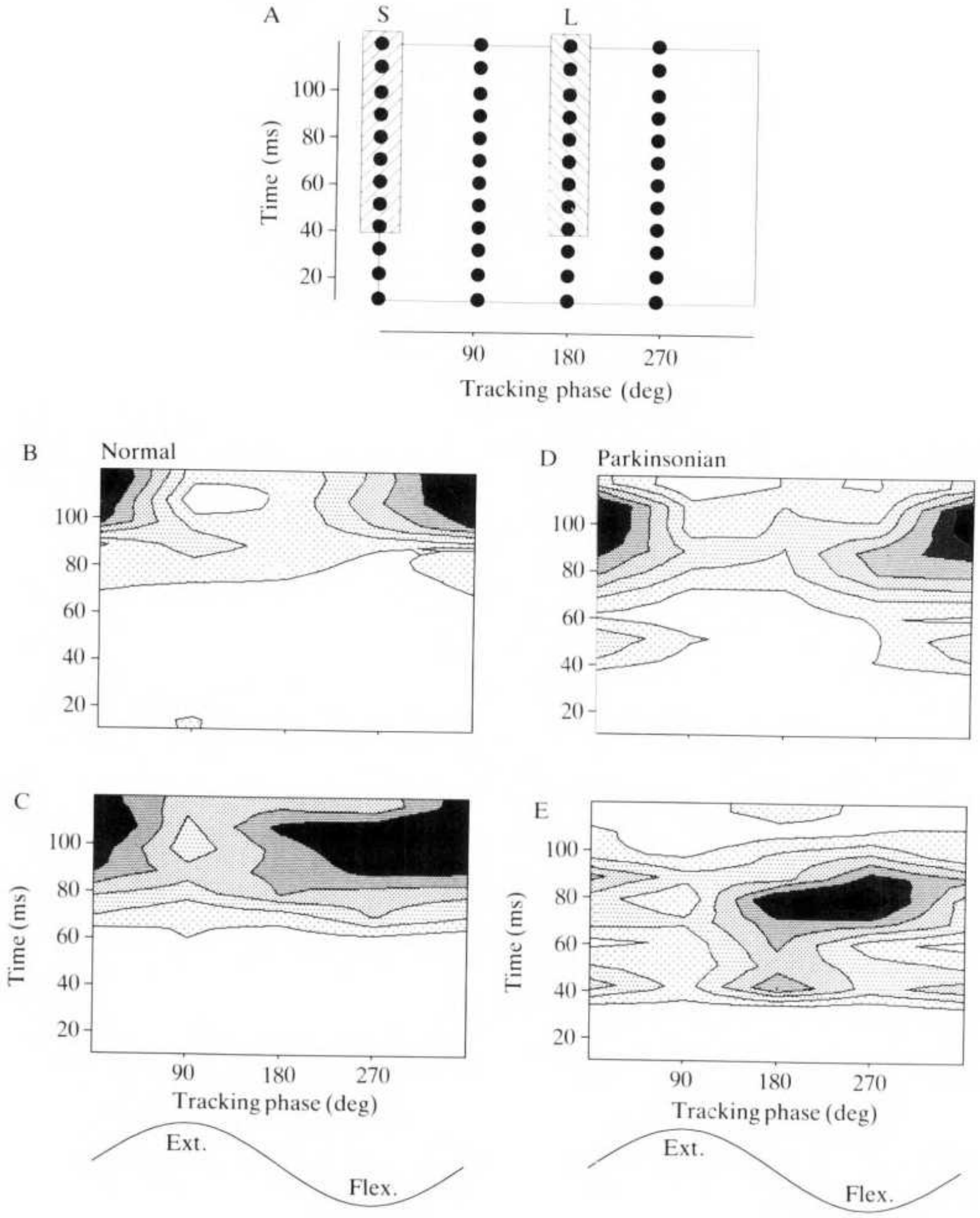

FIG. 6. Modulation of the extensor muscle reflex during sinusoidal tracking. A, diagram of extensor muscle reflex S/L ratio derivation. Hatched elements from 40 to 120 ms indicate the time bins summed to produce the numerator (S) and demoninator (L) of ratio. B, extensor reflex contour plot of the normal with the largest reflex S/L ratio. C, extensor reflex contour plot of the normal with the smallest reflex S/L ratio. D, extensor reflex contour plot of the parkinsonian subject with the largest reflex S/L ratio. E, extensor reflex contour plot of the parkinsonian with the smallest reflex S/L ratio.

intervals used in the reflex S/L index calculation is shown in A. For the extensor, the reflex amplitude data points are subdivided into those measured during maximal tracking velocity into flexion (180°; denoted 'L') and those measured during maximal tracking velocity into extension (0°; denoted 'S'). Extensor reflex contour plots from the normal group, demonstrating the maximum (0.420) and minimum (0.085) reflex S/L ratios are shown in B and C, respectively. Extensor reflex plots from the parkinsonian group, demonstrating the maximum (0.462) and minimum (−0.263) values of the S/L ratio are shown in D and E, respectively. Although the parkinsonian subject with the largest ratio (D) exhibits relatively normal reflex modulation, the subject with the smallest ratio (E) has peak extensor reflex activity between 180° and 270°, phases in which the extensor is lengthening. This profile shows more similarity to a normal flexor muscle reflex profile (fig. 5 A, B) than to a normal extensor reflex profile.

Comparison of the reflex S/L ratio for the wrist flexor between normal and parkinsonian groups is presented in fig. 7. As shown in A, the calculation is analogous to that described for the extensor, except that the numerator 'S' is now calculated at 180° and the denominator 'L' is calculated at 0°. Reflex contour plots for the wrist flexor of the age-matched normals with maximum (1.363) and minimum (0.230) reflex S/L ratios are shown in B and C, respectively. Peak reflex amplitudes are centred around 180°, corresponding to flexor shortening. Flexor muscle reflex contour plots of parkinsonian subjects with the maximum (1.311) and minimum (−0.370) values of reflex S/L ratio are shown in D and E, respectively. In contrast to the normals, the parkinsonian subject (E) demonstrates a reflex modulation with minimal amplitude at 180° (flexor muscle shortening) and greater activity in the region between 270° and 60° (flexor muscle lengthening).

Indices of reflex EMG and volitional EMG modulation from the normal and parkinsonian groups were used to construct fig. 8 and Table 2. The mean extensor reflex S/L ratio (A) is significantly different for the normal and parkinsonian groups ($P = 0.003$). Similarly, comparison of the group means of the flexor muscle reflex S/L ratio (B) yields a significant difference ($P = 0.014$). A worst case S/L ratio (C) consisting of the minimum of the flexor and extensor S/L ratios for a given subject demonstrated highly significant group differences ($P < 0.001$). This confirms the qualitative impression described earlier (*see* fig. 4A, B, fig. 5 C, D) that either the wrist flexor or extensor reflex modulation could be altered in individual subjects with parkinsonism. Modulation indices of volitional EMG for the normal and parkinsonian groups are shown in D−F. The volitional EMG log shortening/lengthening (volitional S/L) ratio is analogous to the reflex S/L ratio, with the difference being the use of volitional EMG obtained in the absence of perturbation. Inspection of all volitional EMG modulation indices (Table 2) reveals no significant difference between groups ($P > 0.6$). The significance of the measured volitional EMG modulation is ascertained by comparing the measured values of the log S/L index to 0, which is the value of the index when $S = L$ (i.e., no detectable modulation). Modulation of the volitional EMG between 0° and 180° is significant ($P < 0.005$) for both flexor and extensor, in both subject groups. Thus the lack of group differences in volitional EMG modulation is not due to insensitive measurement of the EMG signal.

Correlations of clinical symptomatic ratings with the reflex indices are presented in Table 3. New York University disability scale for rigidity and bradykinesia, and the

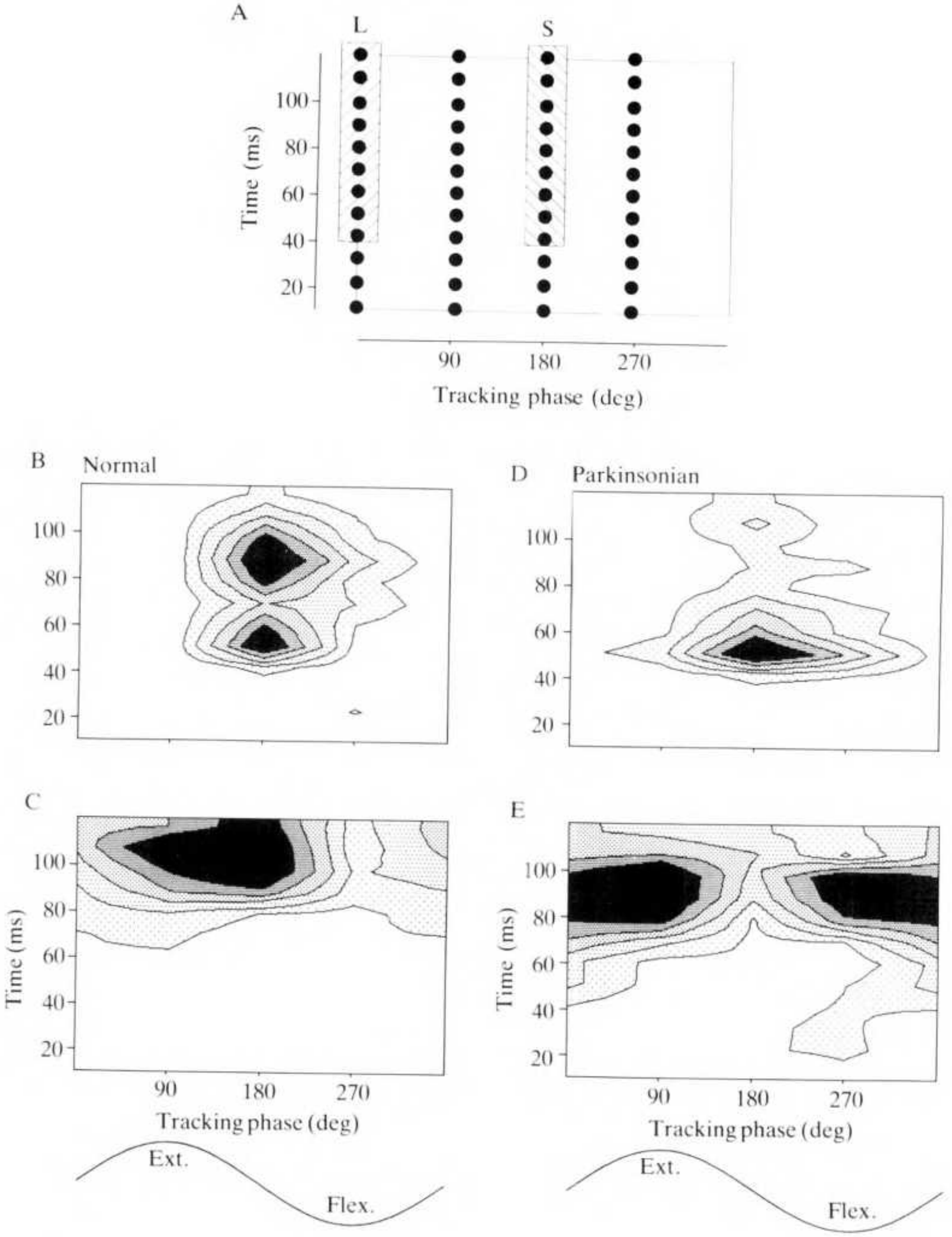

FIG. 7. Modulation of the flexor muscle reflex during sinusoidal tracking. A, diagram of flexor muscle reflex S/L ratio derivation. Hatched elements of EMG array indicate source of numerator (S) and denominator (L) of ratio. B, flexor muscle reflex contour plot of the normal with the largest reflex S/L ratio. C, flexor muscle reflex contour plot of the normal with the smallest reflex S/L ratio. D, flexor muscle reflex contour plot of the parkinsonian subject with the largest reflex S/L ratio. E, flexor muscle reflex contour plot of the parkinsonian subject with the smallest reflex S/L ratio.

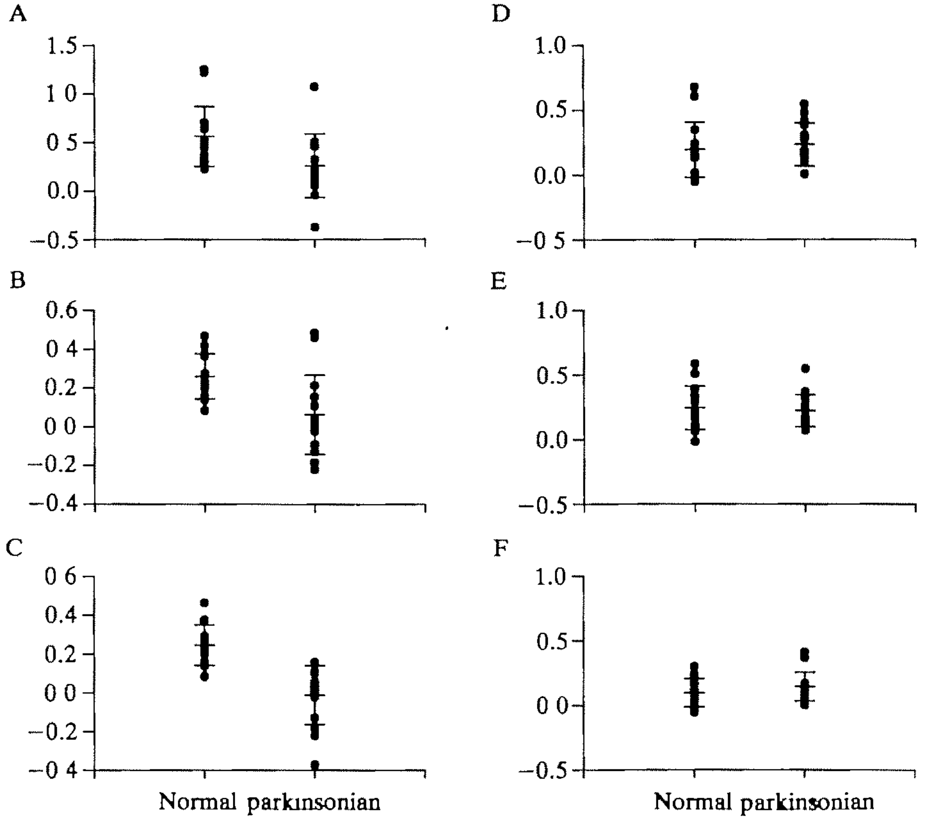

FIG. 8 Modulation indices for reflex (*left column*) and volitional (*right column*) EMG A–C, group comparisons of reflex EMG indices, consisting of flexor S/L ratio, extensor S/L ratio, and worst case S/L ratio. D–F, group comparisons of volitional EMG indices, consisting of flexor S/L ratio, extensor S/L ratio, and worst case S/L ratio Data points are filled circles Bars indicate mean ± SD.

TABLE 2 MODULATION AND COACTIVATION INDICES

	Normal		*Parkinsonian*		*t test*
	Mean	*SD*	*Mean*	*SD*	*P value*
Reflex shortening/lengthening indices					
Flexor R S/L	0.56	0.31	0.26	0.33	0.014
Extensor R S/L	0.26	0.12	0.06	0.21	0.003
Worst R S/L	0.25	0.10	−0.01	0.15	<0.001
Volitional shortening/lengthening indices					
Flexor V S/L	0.26	0.21	0.23	0.17	0.595
Extensor V S/L	0.24	0.17	0.22	0.12	0.681

Hoehn and Yahr scale for global disability stage were used (for review, *see* Martínez-Martín and Bermejo-Pareja, 1988). As clinical ratings for rigidity and bradykinesia are ordinal in nature, the nonparametric Spearman rank correlation was used to compare the clinical and physiological evaluations (Mackenzie and Charlson, 1986). The results of the physiological testing were not made known to the clinical evaluator during the

TABLE 3. CORRELATION OF REFLEX MODULATION INDICES AND CLINICAL RATINGS

	F R S/L	*E R S/L*	*Bradykinesia*[1]	*Rigidity*[2]
E R S/L	−0.05			
Bradykinesia[1]	−0.68**	−0.35		
Rigidity[2]	−0.38	−0.23	0.58*	
Stage[3]	−0.60*	−0.32	0.81**	0.65**

* $P < 0.05$ (Spearman rank correlations); ** $P < 0.02$. F R S/L = reflex log shortening/lengthening index of flexor; E R S/L = reflex log shortening/lengthening index of extensor. [1] Clinical bradykinesia rating. [2] Clinical rigidity rating. [3] Hoehn and Yahr rating scale.

study. Significant correlations of physiologically derived indices and clinical ratings occur between the flexor muscle reflex S/L and bradykinesia ($P < 0.02$) and stage ($P < 0.05$) ratings. No significant ($P < 0.05$) correlations with the clinical ratings were found for the extensor reflex S/L index. However, a trend of decreasing extensor reflex S/L ratio with increasing bradykinesia rating is present (mean S/L ratio at a rating of 0 bradykinesia is 0.12, mean S/L ratio at ratings of 2−4 bradykinesia is −0.01). The Hoehn and Yahr composite stage index correlates well to the rigidity and bradykinesia ratings, indicating internal consistency in the clinical rating.

DISCUSSION

The results of the present experiment demonstrate that the link between reflex and volitional modulation of EMG can be altered in pathological states. A significantly altered modulation of the long-latency stretch reflex in subjects with Parkinson's disease was observed, with peak reflex activity occurring in phases of movement corresponding to homonymous muscle lengthening. In cases of parkinsonism, the reflex modulation envelope may shift by as much as 180° (2 s of the tracking period), while the volitional EMG modulation remains correctly situated. The altered reflex modulation occurs with the tracked wrist movement and associated volitional EMG remaining normal. It should be stressed that this is not a statement of normality of the volitional system in parkinsonism. In the present paradigm, a stereotyped tracking performance within specified error limits is required, and thus the tracked wrist movement and volitional EMG were constrained.

The altered long-latency reflex modulation in parkinsonism during tracking is seen as an impediment to volitional movement, contributing to bradykinesia. The normal increase in the reflex activity during homonymous muscle shortening serves to produce torque which augments the volitionally generated torque, in accord with the principle of servo-assistance (Marsden *et al.*, 1972, 1976). When reflex activity shifts to phases of movement in which homonymous muscle lengthening occurs, the reflex-generated torque opposes the joint movement (Liddell and Sherrington, 1924; Gottlieb and Agarwal, 1973). In normals, reflex gain is minimal at this tracking phase. The reflex modulation index (log of ratio of reflex EMG activity during shortening to activity during lengthening) may be interpreted as a ratio of 'assistive' reflex activity to 'braking' reflex activity, relative to the sinusoidal tracking. Index values above zero signify that the reflex exerts comparatively more activity in phases of movement which augment the volitionally generated torque. Values of the index below zero indicate that the reflex exerts comparatively more activity in phases of movement in which the reflex opposes the volitionally generated torque. The sample of parkinsonian subjects has a mean of -0.01 for the worst case reflex S/L index (Table 2, fig. 8), with 6 subjects having negative values. In contrast, all the normal subjects have positive individual reflex S/L indices and a mean value of $+0.25$ for the worst case reflex S/L index (Table 2, fig. 8).

A further link between the changes in reflex modulation in parkinsonism and the degree of bradykinesia arises from the correlation of the reflex modulation indices with the clinical ratings. Parkinsonian subjects with clinical ratings of 2 or greater, using the NYU bradykinesia subset, all had flexor, extensor, and the worst case reflex S/L indices smaller than any member of the normal group. Trends of decreasing reflex S/L ratios as a function of the NYU bradykinesia subset exist for both the flexor and extensor. The flexor muscle reflex S/L index inversely correlates with the ratings of bradykinesia ($P < 0.02$) and total disability ($P < 0.05$). The lack of significant correlation of the clinical ratings with the extensor reflex S/L index may reflect an alteration in the flexor-extensor postural balance noted in parkinsonism. Denny-Brown (1962) described a progressive course of upper extremity flexion dystonia. There exist multiple reports of upper extremity flexor drift after lesioning of the basal ganglia in primates (DeLong and Coyle, 1979; Hore and Vilis, 1980).

Most studies documenting an increase in the long-latency reflex amplitude in parkinsonism (Tatton and Lee, 1975; Marsden *et al.*, 1978; Mortimer and Webster, 1979; Berardelli *et al.*, 1983; Rothwell *et al.*, 1983; Cody *et al.*, 1986) relate this observation to the symptom of rigidity. It has been postulated that rigidity may be caused by a long-latency reflex with gain constantly set at high levels (Lee and Tatton, 1975; Mortimer and Webster, 1979). However, in the detailed studies by Rothwell *et al.* (1983) and Cody *et al.* (1986), the correlation of the long-latency reflex response to clinical impressions of rigidity was imperfect. Interestingly, the changes in reflex modulation observed in this study were not correlated with the clinical ratings of rigidity, but were correlated with the degree of bradykinesia.

Bradykinesia is a broadly descriptive term, and it is unlikely that a single mechanism will explain all aspects of the condition (Delwaide and Gonce, 1988). Hypothetical mechanisms of disordered motor control in bradykinesia have included defects in the execution of central motor programs (for review, *see* Marsden, 1982; Delwaide and Gonce, 1988). The hypothesis of a defect in the processing of central motor programs in bradykinesis arose initially from reaction and movement time studies (Brumlik and Boshes, 1966; Evarts *et al.*, 1981). Also, the abnormal character of agonist-antagonist EMG sequencing during ballistic movements in parkinsonism has been interpreted as resulting from a defect in execution of central motor sequence programs (Hallett and Khoshbin, 1980). Similar abnormalities in the agonist-antagonist EMG sequencing were observed for fast planar geometric figure tracing (Berardelli *et al.*, 1986). The above evidence for defective central program execution consists of movement and volitional EMG sequencing that deviate from normal. Since the present data were recorded only when tracking was within acceptable error margins, the present paradigm underestimates the component of bradykinesia arising from faulty processing of central motor programs.

Changes in alpha motoneuron activity and reciprocal inhibition have also been proposed as contributing to bradykinesia (Delwaide and Gonce, 1988). Enhanced supraspinal drive on alpha motoneuron activity, as evidenced by increased resting EMG levels, has been reported in parkinsonism (Marsden, 1982; Berardelli *et al.*, 1983). This mechanism may retard the intended volitional movement if it results in a coactivation of antagonistic muscles. However, there is little overlap between agonist and antagonist EMG for ballistic elbow flexion, suggesting that reciprocal inhibition is normal (Hallett and Khoshbin, 1980). Also, Hagbarth *et al.* (1975) noted that tremor produced reciprocal EMG patterns in antagonistic muscles. Volitional EMG modulation and the amount of coactivation (judged by depth of modulation) did not differ between groups for the slow tracking movement used in this study. The argument that a disordered volitional EMG modulation produced the change in the reflex modulation via a linkage as decribed by the automatic gain hypothesis (Marsden *et al.*, 1972) is weakened by four observations. First, the morphology of the volitional EMG envelope is similar to that for normal subjects (fig. 1). Secondly, the amount and direction of volitional EMG modulation over phase is the same for both groups (fig. 8, Table 2). Thirdly, the amount of volitional EMG at the perturbation assay points, normalized to the mean across tracking phase, is the same for both groups (flexor at 0°: $P > 0.6$, flexor at 180°: $P > 0.5$, extensor at 0°: $P > 0.2$, extensor at 180°: $P > 0.4$). Finally, since a movement requires increased muscle activation and EMG during shortening above that present during lengthening, the peak of reflex amplitude occurring during lengthening cannot correspond to peak volitional EMG amplitude (*see* fig. 4A, C, fig. 5D, E, fig. 6E, fig. 7E).

The evidence presented points to a violation of servo-assistance and the automatic gain hypothesis in the parkinsonian group. This finding is potentially at odds with the observation that alpha-gamma linkage has been demonstrated to be intact in parkinsonian patients during tremor (Hagbarth *et al.*, 1975) and fluctuations of rigidity (Wallin *et al.*, 1973) by single and multiple unit recording. Those findings, however, have been limited to muscle contractions measured about an isometrically positioned joint. The modulation of reflex magnitude during sinusoidal tracking movements may be due to supraspinal gamma control, descending influences on spinal circuitry, or central influences on transcortical reflex loops. Although the exact mechanisms remain to be elucidated, if the reflex amplitude modulation is the result of supraspinal gamma control, this would suggest that some alteration of alpha-gamma linkage does occur during volitional movements in parkinsonism. The observed change in reflex modulation during volitional tracking has relevance to arguments of the dependence or independence of the supraspinal reflex control system and volitional control system (Gottlieb *et al.*, 1973; Stein, 1974; Dufresne *et al.*, 1980; Soechting *et al.*, 1981). In this controversy, the relationship between the stretch reflex amplitude modulation and the volitional EMG modulation has been of considerable interest. Dissociation of reflex and volitional activities argues against a rigid linkage of the two systems.

ACKNOWLEDGEMENTS

We are indebted to the patients who consented to testing. Michael McPhee provided assistance with graphics. The work was funded by EMPI Inc., American Parkinson Disease Association, Minnesota Medical Foundation, and Mr Hal Seth.

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(Received November 25, 1989. Revised February 14, 1990. Accepted March 20, 1990)

Brain (1991), **114**, 461–472

MICROSACCADIC FLUTTER

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SUMMARY

Microsaccadic flutter is a rare symptomatic saccadic oscillation that has been reported only twice previously. Here we describe 5 patients with this disorder. The oscillation is horizontal, has a frequency of 15–30 Hz, an amplitude of 0.1–0.5°, and cannot be seen with the unaided eye. It is usually not associated with any underlying neurological disorder. We hypothesize that microsaccadic flutter is due to malfunction of the brainstem omnipause neurons.

INTRODUCTION

Saccadic oscillations, composed of back-to-back saccades, may be found in normal human beings as well as in patients with neurological disorders. Such saccadic oscillations are composed of multiple consecutive oppositely-directed saccades, without an intersaccadic interval. This last feature distinguishes them from the common 'square wave jerk' oscillation, in which there is typically an intersaccadic interval of about 200 ms.

Saccadic oscillations made up of back-to-back saccades can be subdivided into three forms. The first is *voluntary nystagmus*: some individuals have the ability to generate bursts of high frequency oscillations of approximately 2–5° in amplitude (Shults *et al.*, 1977; Zahn, 1978). Although clear vision is impossible during the nystagmus, voluntary nystagmus is not troublesome because it is initiated voluntarily.

The two symptomatic saccadic oscillations are opsoclonus and ocular flutter. *Opsoclonus* was first described by Orzechowski (1927) as a 'chaotic, unpredictable', 'great agitation of the eyes'. It consists of large-amplitude randomly-directed chaotic saccades occurring in the horizontal, vertical and torsional planes. *Flutter* consists of bursts of back-to-back horizontal saccades, about 1–5° in amplitude, often precipitated by a change in gaze. Opsoclonus and flutter are closely related and may be seen in the same patient during the course of an illness. Orzechowski originally described opsoclonus in 6 patients with epidemic encephalitis and even now the most consistent association is brainstem encephalitis (Digre, 1986). Opsoclonus and flutter are also commonly a paraneoplastic effect of neuroblastoma and tumours of the lung, breast and uterus (Orzechowski, 1927; Smith and Walsh, 1960; Cogan, 1968; Ellenberger *et al.*, 1968; Rivner *et al.*, 1982; Kuban *et al.*, 1983; Digre, 1986; Anderson *et al.*, 1988). A number of other associations have been reported (Leigh and Zee, 1991).

Here we report 5 patients with a distinctive symptomatic saccadic oscillation that we call *'microsaccadic flutter'*. These patients complained of impaired vision and, although their eye movements were normal to visual inspection, all had abnormal microsaccadic

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oscillation detectable by ophthalmoscopy and by high-resolution eye movement recordings. Microsaccadic flutter was associated with an underlying neurological disorder (multiple sclerosis) in only 1 of 5 patients.

METHODS AND SUBJECTS

We recorded horizontal and vertical eye movements with a scleral annulus using the magnetic field-search coil technique. This method has a sensitivity of about 0.004° (Robinson, 1963; Collewijn *et al.*, 1975). Blinks were detected by electro-oculography (EOG) using a pair of electrodes placed above and below the right eye. The bandwidth of the recording apparatus was limited by the chart recorder (Gould model 2400) to 0–60 Hz. For comparison with our patients we recorded saccades in 7 normal subjects. These saccades were elicited by 1° steps of a LED target array, and analysed by procedures discussed in detail elsewhere (Kapoula *et al.*, 1986).

CASE REPORTS

Case 1

A 20-yr-old woman presented with a 5 yr history of spells of 'shimmering' vision. The spells occurred daily, each lasting minutes to hours. They were initially associated with headaches but continued to occur every day after the headaches were relieved by propranolol. Shimmering could be precipitated by bright lights and also occurred spontaneously. The neurological examination was normal except for ophthalmoscopy which revealed an intermittent high-frequency oscillation in both eyes. Fig. 1A illustrates a 30 Hz oscillation that ranged in amplitude from 0.1° to 0.25°.

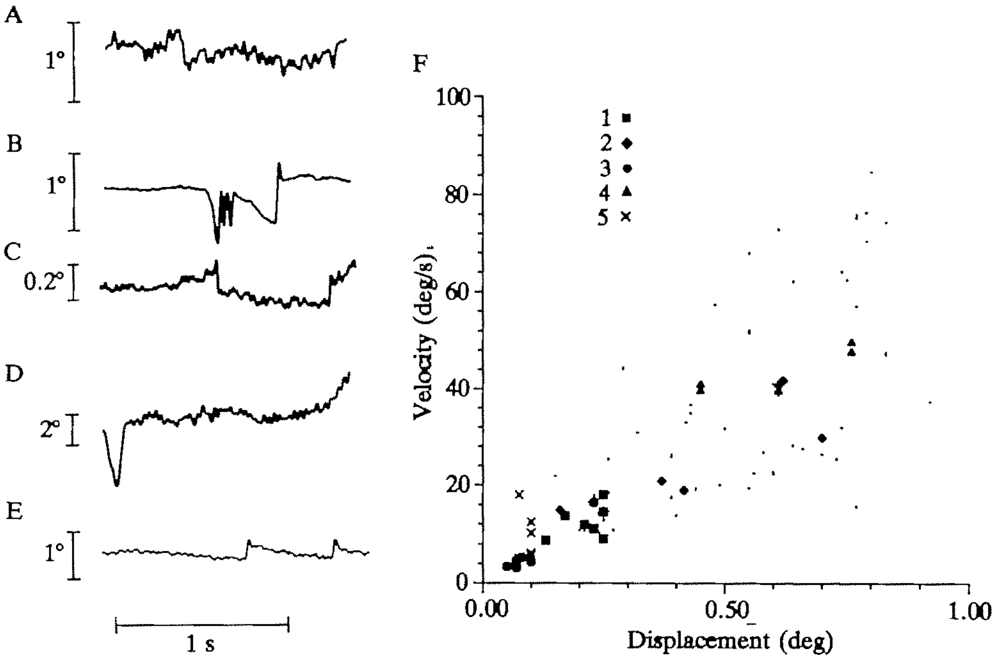

FIG. 1A–E. Microsaccadic flutter in *Cases 1–5* (F) Main sequence plot of patients and 7 normal subjects. Data from normal subjects are plotted as dots. Data from patients are plotted as symbols indicated in the key. The movements of all our patients had the same peak-velocity relationship as did the normal saccades.

Computed tomography of the head and auditory-evoked responses were normal Clonazepam improved her symptoms. Three years later she was asymptomatic, taking no medication.

Case 2

A 29-yr-old woman presented with a 3 yr history of visual symptoms. These were described as 'jiggling' of her visual field during which she felt everything about her was moving. Viewing out of either eye was equally troublesome. The episodes could last up to an hour and occurred almost daily. The patient felt that these symptoms were triggered and exacerbated by anxiety.

The neurological examination was normal except for ophthalmoscopy which revealed saccadic oscillations. Fig 1B illustrates bursts of 20 Hz saccadic oscillation with an amplitude ranging from 0.3° to 0.8°. They were usually accompanied by a blink. Results of an MRI scan of the head, lumbar puncture, auditory and visual-evoked potentials, chest x-ray and a gynaecological examination were normal. Clonazepam, propranolol and verapamil all reduced her visual symptoms but were accompanied by unacceptable side-effects Two years later her symptoms and examination were unchanged.

Case 3

A 36-yr-old man gave a 3 yr history of dizziness and a 6 month history of an intermittent visual disturbance, both described as a 'wavy' sensation. The visual disturbance occurred several times each hour and lasted for a few seconds. Head motion and physical activity provoked these symptoms Neurological examination was normal except for ophthalmoscopy which showed a saccadic oscillation in both eyes. Fig. 1C shows a 20 Hz saccadic oscillation with an amplitude of 0.05°. An MRI scan of the brain and auditory-evoked responses were normal. The CSF contained no cells but the protein concentration was slightly increased at 0.54 g/l.

Case 4

A 45-yr-old man presented with a 6 month history of vertigo, nausea, blurred vision and disequilibrium. When viewing nearby objects he noted a decrease in his visual acuity and 'shimmering'. These symptoms began immediately after the removal from the stomach of an inflatable silastic balloon which had been placed for the treatment of obesity. Neurological examination was normal except for ophthalmoscopy which revealed an intermittent saccadic oscillation in both eyes. Fig. 1D shows a 20–25 Hz saccadic oscillation with an amplitude of about 0.5°. The oscillation could be induced by horizontal pursuit. An MRI of the brain was normal as was computed tomography of the thorax and abdomen.

Case 5

A 45-yr-old woman presented with a 2 yr history of visual disturbance that she described as 'laser beams shooting out from lights'. Her symptoms occurred daily and were associated with unsteadiness and tinnitus. General neurological examination was normal except for ophthalmoscopy which revealed bursts of low-amplitude high-frequency eye movements in both eyes. Fig. 1E illustrates a 15 Hz saccadic oscillation with an amplitude of 0.1°. This sample is part of a burst of nystagmus that lasted 30 s.

An MRI scan showed multiple periventricular lesions in the white matter. Examination of the spinal fluid was normal at the time of presentation but 1 yr later showed oligoclonal IgG bands. Multiple sclerosis was diagnosed. Her symptoms did not improve with carbamazepine. On follow-up examination 3 yrs later she still had the visual complaints.

DISCUSSION

These 5 patients had a symptomatic ocular oscillation consisting of horizontal back-to-back saccades. The oscillation had a frequency ranging from 15 to 30 Hz and an amplitude ranging from 0.1 to 0.5°. The oscillations usually could not be detected by direct visual inspection. The typical patient was young and in otherwise good health. The symptoms were usually disturbing rather than disabling and were often described in an unusual way. Our patients used the words 'shimmering' or 'jiggling', 'wavy', and 'laser beams'. The visual disturbance would last for seconds to hours and often occurred daily. Dizziness or disequilibrium often accompanied the visual symptoms. The only abnormality on neurological examination was the saccadic oscillation.

Microsaccadic flutter has received little attention. Sharpe and Fletcher (1986) reported a patient with a cerebellar degeneration who had a similar entity, which they called 'microflutter'. They described an irregular low-amplitude oscillation of approximately 20 Hz, preceded by a train of double saccadic pulses. Carlow (1986) reported a patient with a long history of oscillopsia who had back-to-back saccades with an amplitude of approximately 1°. These oscillations, also called 'microflutter' by Carlow, could only be seen with an ophthalmoscope. In this case no information was given regarding an underlying neurological disorder. Although the above authors used the term 'microflutter', we prefer the more specific term 'microsaccadic flutter'.

Differential diagnosis

There are two nonsaccadic ocular oscillations that must be differentiated from microsaccadic flutter. Superior oblique myokymia may also present with intermittent visual obscuration. It occurs in otherwise healthy individuals, and may require magnification in order to be appreciated (Susac *et al.*, 1973). Unlike microsaccadic flutter, superior oblique myokymia is always monocular and has a strong torsional component.

Ocular microtremor may also resemble microsaccadic flutter. Ocular microtremor can be recorded in normal subjects during steady fixation. It is a continuous near-sinusoidal oscillation that has a frquency of 100 Hz and a mean amplitude of 30 s of arc (about 0.008°) (Coakley, 1983). Microsaccadic flutter is larger in amplitude (0.1−0.5°) and lower in frequency (15−30 Hz). Ocular microtremor of a lower frequency has been described in patients with alteration in their level of consciousness (Coakley, 1983), but our patients were alert when examined and tested.

Microsaccadic flutter must also be differentiated from voluntary nystagmus and opsoclonus. Patients with voluntary nystagmus can control their saccadic oscillations and consequently their symptoms. Although hysterics and malingerers may have the ability to produce voluntary nystagmus that resembles flutter, voluntary nystagmus can be distinguished from microsaccadic flutter by the usual accompaniments of a 'fixed look' required to produce the symptoms, convergence, and an oscillation that is easily visible to the naked eye (Hotson, 1984). Opsoclonus is easily distinguished from microsaccadic flutter by its large amplitude and multidirectionality.

It might be argued that microsaccadic flutter is merely a smaller-amplitude variant of ocular flutter. There may, however, be an advantage in distinguishing between microsaccadic flutter and the other symptomatic oscillations because they may reflect differing underlying mechanisms and pathology. Both opsoclonus and flutter are usually associated with an underlying neurological disorder. On the other hand, in only 1 of our patients (Case 5 who developed multiple sclerosis) was there a neurological condition that might be related to the saccadic oscillation. Furthermore, we shall subsequently argue that the pathophysiology of flutter and microsaccadic flutter differs.

Pathophysiology

It will be useful to consider first the neural network that generates saccades—the so-called saccadic pulse generator. For horizontal saccades the saccadic pulse generator is comprised of three major groups of interconnected neurons (fig. 2). *Excitatory burst neurons* (EBN) discharge in proportion to eye velocity and drive the ocular motoneurons during a saccade. They are found just above the abducens nuclei in the paramedian pontine reticular formation (PPRF). *Omnipause neurons* (OPN) inhibit the burst cells between saccades and must themselves be inhibited to allow a saccade to proceed. Omnipause neurons are found between the abducens nuclei in the nucleus raphe interpositus (Büttner and Büttner-Ennever, 1988). The *neural integrator* (NI) is comprised of neurons that provide a tonic signal proportional to eye position and so provide innervation needed to maintain eccentric position of gaze after the saccade is over. The neural integrator for horizontal movements is found just below the abducens nucleus in the nucleus prepositus hypoglossi (nph) and medial vestibular nuclei. It should be noted that most of the neurons in the neural integrator also discharge in proportion to eye velocity during saccades. The neural integrator is thought to be distributed over a network of burst-tonic neurons (Cannon and Robinson, 1985).

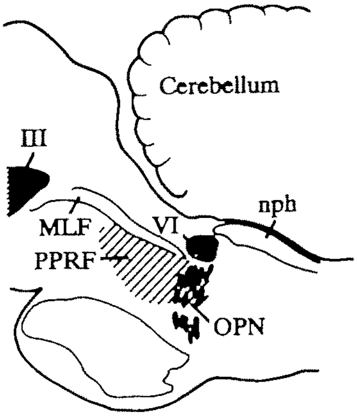

FIG 2. A sagittal section of the monkey brainstem (after Buttner and Buttner-Ennever, 1988) to show the location of cell groups thought to comprise the saccadic pulse generator. The shaded area indicates the paramedian pontine reticular formation (PPRF) which contains the horizontal excitatory burst neurons. The omnipause (OPN) are found between the rootlets of the abducens nucleus (VI) in the raphe nucleus interpositus Neurons that discharge in proportion to eye position are found in the nucleus prepositus hypoglossi (nph) and medial vestibular nucleus (not shown).

In patients with saccadic oscillations, lesions might be expected in one or more of the three groups of neurons discussed above, and specifically, close to the pontomedullary junction. Contrary to this supposition, autopsy studies of patients with opsoclonus generally show lesions in the cerebellum, in the diencephalon, or diffusely in the brainstem. The Table summarizes 23 reported autopsies of cases with opsoclonus; 12 cases showed some involvement of the cerebellum.

The mechanism by which cerebellar lesions might cause saccadic oscillations is unclear. Physiological and lesion studies have shown that the cerebellum is important for determining the accuracy of saccades but that it is not essential for the generation of saccades (Westheimer and Blair, 1973; Ritchie, 1976; Optican and Robinson, 1980).

TABLE REPORTS OF AUTOPSIED CASES OF OPSOCLONUS

Authors	*Cerebellum*	*Brainstem*	*Diencephalon*	*Cortex*
Alessi (1940)	x			
Ellenberger *et al.* (1968)	x			
Moe and Nellhaus (1970)	x			
Ziter *et al.* (1979)	x			
Kearsley *et al.* (1985)	x			
Hunter and Kooistra (1986)	x			
Estrin (1977)	x	x		x
Ross and Zeman (1967)	x	x		
Turazzi *et al.* (1977)	x	x		x
Graus *et al.* (1984)	x	x		
Kilgo and Schwartze (1984)	x			
Ridley *et al.* (1987)	x	x		
Cogan (1954)		x	x	
Kennedy and Tucket (1962)		x	x	
Keane (1980)		x	x	
Lechi *et al.* (1983)		x	x	
Keane and Devereaux (1974)		x	x	x
Wertenbacker *et al.* (1981)		x	x	x
Anderson *et al.* (1988) Case 1		x	x	x
Case 2		x	x	x
Ridley *et al.* (1987)		(Normal)		
Lemerle *et al.* (1969)		(Normal)		
Lipinski *et al.* (1975)		(Normal)		

Furthermore, experimental cerebellar lesions have not been reported to cause saccadic oscillations without an intersaccadic interval. Although clinical reports have been interpreted as indicating that saccadic oscillations can be due to cerebellar lesions alone (Ellenberger *et al.*, 1968; Ziter *et al.*, 1979) there must be other causes since no cerebellar lesions were found in almost half of the reported cases. Nevertheless, since the cerebellum is intimately connected with the brainstem circuits that generate saccades it could be speculated that inappropriate cerebellar discharges might lead to opsoclonus or flutter.

What about a brainstem localization for saccadic oscillations? Lesions of the posterior commissure or bilateral lesions of the pretectum cause 'lightning eye movements' in monkeys (Pasik *et al.*, 1969). Pasik *et al.* described the movements that were observed experimentally as similar to opsoclonus: 'as if the affected globe were made of a gel which was suddenly jarred'. Eye movement recordings of these oscillations, however, showed both slow and fast phases rather than the back-to-back saccades that are the hallmark of opsoclonus.

Some clinical reports do, however, point to a brainstem localization. Of the reported autopsies, 13 showed diffuse involvement of the brainstem. Keane reported opsoclonus in 2 patients with presumed midbrain lesions (Keane and Devereaux, 1974; Keane, 1980) and there are reports of opsoclonus in cases with lesions in adjacent structures such as the hypothalamus (Cogan, 1954; Kennedy and Tucket, 1962; Wertenbacker, 1981; Lechi *et al.*, 1983; Anderson *et al.*, 1988). Unfortunately, in most of these cases the exact nature of the oscillation was not documented with eye-movement recordings. It is possible, for example, that in some of these cases convergence-retraction nystagmus was mistaken for opsoclonus. Recently it has been proposed that the superior colliculus might also be a part of the saccadic pulse generator (Waitzman *et al.*, 1988). If this were the case, then midbrain lesions that disturbed the superior colliculus might also lead to saccadic oscillations.

Taking into account the results of both the experimental work and the clinical case reports it seems reasonable to conclude that saccadic oscillations can arise both from intrinsic lesions of the pulse generator and from abnormal inputs to the pulse generator including those from the midbrain, diencephalon and probably the cerebellum.

Finally, for the purpose of distinguishing microsaccadic flutter from other saccadic oscillations it will be useful to consider current analytical models of saccadic generation and the inherent predisposition of the saccadic system towards instability. The interconnections between the neurons known to participate in saccadic control are shown in fig. 3. The combination of connections and neurons in the saccadic pulse generator forms a neural servomechanism that probably works in the following way. When a saccade is desired to bring the eyes to a new orbital position, a command signal (EΔ) encoding the desired change in eye position is generated and passed on to excitatory burst neurons (EBN) as a motor error signal. Pause cells (OPN), which are kept discharging by a tonic bias (TONE), are inhibited at the same time by a trigger signal (TRIG). EBN neurons are stimulated by the motor error signal. They move the eyes by direct projections to ocular motoneurons (OMN), and provide an input to the neural integrator for the generation of the tonic signal to hold the eye in position at the end of the saccade. EBN also drive inhibitory burst neurons (IBN) which in turn inhibit pause cells during the saccade. The EBN neurons are also thought to project to a separate, resettable (after each saccade), neural integrator (RI) that generates an internal representation of the actual change in the position of the eye. This signal is then fed

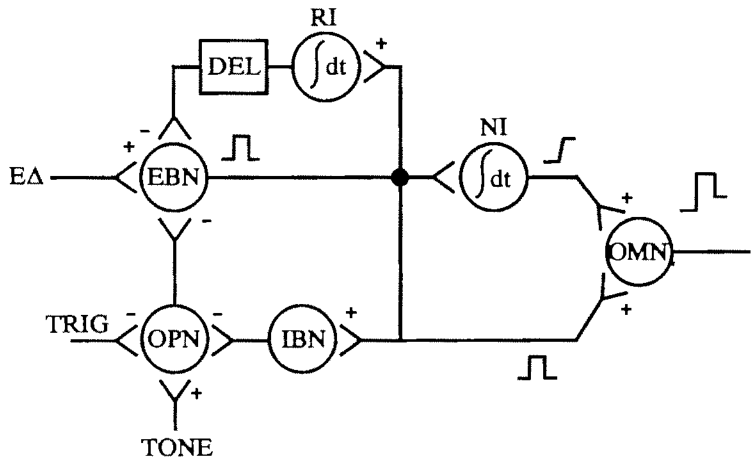

FIG. 3 The neural circuitry that underlies saccade generation. Neuronal elements include excitatory burst neurons (EBN), inhibitory burst neurons (IBN), tonic neurons in the neural integrator (NI) and omnipause neurons (OPN). Input signals include desired change in eye position (EΔ) and a trigger signal (TRIG). TONE (nominal value 30) is the bias to the omnipause neurons. RI is the resettable integrator. ∫ dt denotes integration over time. DEL (nominal value 10 ms) indicates the delay between the output of RI and the EBN OMN are the ocular motor nuclei.

back and subtracted from the desired change in eye position. When these two signals become equal, i.e., the eye has reached the target, the motor error signal driving the burst neurons becomes zero, the burst neurons cease discharging and the saccade is over. While hypothetical, the essence of this scheme has considerable support in both theory and the experimental evidence (Zee and Robinson, 1979; Jürgens *et al.*, 1981; Scudder, 1988).

Crucial to understanding oscillations is recognition of the intrinsic biological delays associated with propagation of impulses along neural pathways. For example, it may take 8–10 ms for signals to traverse the upper feedback loop of fig. 3. Thus while the feedback mechanism of fig. 3 accounts for the ability of the pulse generator to produce saccades of approximately the correct size, there is nonetheless an overshoot (due to the delay) and a consequent susceptibility to endpoint oscillations (small, back-to-back saccades following the initial saccade). The amplitude and frequency of such oscillations will depend upon the duration of the delay and the level of excitability or gain (discharge rate/motor error) of saccadic burst neurons.

The lower portion of fig. 3 contains neurons and connections that function to stabilize the upper loop. It is thought that the pause neurons switch the upper loop off after a saccade has been completed. In this way, the saccadic system can respond rapidly to a new desired eye position signal, yet the eye can remain still after the desired change in position has been completed.

The crucial role of the pause cells as stabilizers of an inherently unstable saccadic generator has recently been questioned. Kaneko and Fuchs (1987) reported that experimental lesions of the pause cells made in monkeys with kainic acid caused slow saccades but not saccadic oscillations. Whether or not the lesions affected all of the pause cells and spared the burst neurons close by is not known. Scudder (1988) proposed an analytical model of the saccadic system in which so-called long-lead burst neurons play a central role. His model responds to a pause cell lesion by producing slow saccades rather than saccadic oscillations (Kaneko, 1989). Scudder's model, however, does not reproduce the saccadic oscillations that do occur in both normal human subjects and in patients. Thus models of the type shown in fig. 3 remain a viable conceptual scheme for analysing human saccadic function.

Zee and Robinson (1979) pointed out that an analytical model incorporating the above connections can be used to identify where lesions could result in saccadic oscillation. The two types of lesion that cause saccadic oscillations are ones that increase the intrinsic instability of the upper portion of the circuit (due to increases in the delay in the feedback loop or in the gain of the burst cells) or ones that impair the function of the lower, stabilizing portion of the circuit.

If the pause cells are removed or disconnected from the other neurons in the simulation, the model of fig. 3 develops a continuous oscillation, having a small-amplitude, high-frequency waveform (*see* upper trace of fig. 4) similar to that seen in voluntary nystagmus and in our patients with microsaccadic flutter. The oscillations of flutter and opsoclonus, however, are lower in frequency and larger in amplitude than those produced by removing the pause cell input alone. To simulate flutter and opsoclonus one must not only decrease the level of pause cell activity but also increase the delay in the upper loop of fig. 3. This type of oscillation is shown in the lower trace of fig. 4. As the delay is increased,

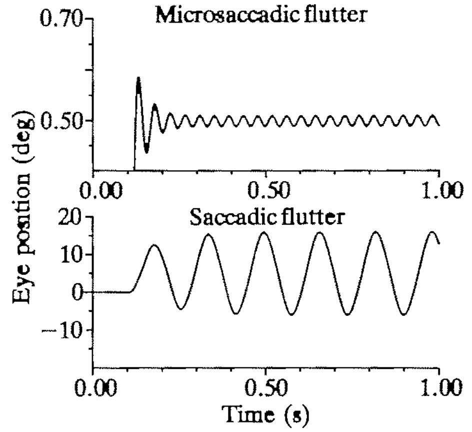

FIG. 4. Model simulation. The *upper trace* shows microsaccadic oscillation caused by reducing the TONE (*see* fig 3) to the pause cells to zero. The *lower trace* shows the larger amplitude and lower frequency saccadic oscillation caused by both reducing pause cell tone to zero and increasing the internal delay (DEL) (*see* fig 3) to 40 ms.

the amplitude of the oscillation increases and the frequency decreases. If the delay is made large enough, oscillations can be sustained even when the level of pause cell tone is normal. Also, when the delay is set to a value intermediate between normal and the value required for sustained oscillation, damped oscillations can be produced after a saccade terminates. This may explain why flutter often occurs immediately after a voluntary saccade (Cogan, 1954). The concept that flutter or opsoclonus might be possible even if pause cell function were normal is also consistent with the pathological study of Ridley *et al.* (1987). They reported 2 patients with opsoclonus in whom injury to the pause cells was not identified.

This analysis suggests that there may be two distinct mechanisms for saccadic oscillation, one which causes large oscillation such as opsoclonus or flutter, and another which causes small oscillations such as voluntary nystagmus or microsaccadic flutter. Flutter or opsoclonus might require an increase in the delay of the feedback signal used by the saccadic pulse generator to terminate the saccade. On the other hand, small-amplitude oscillations might be provoked simply by lowering the tone in the pause cells. The amplitude and frequency would depend upon the inherent values of the delay in the pulse generator and the excitability (or gain) of the burst cells. Lowering the tone in the pause cells might account for the deliberate production of saccadic oscillations known as voluntary nystagmus, or may occur involuntarily and be the cause of microsaccadic flutter.

In summary, microsaccadic flutter consists of back-to-back saccadic oscillations, not visible by unaided inspection but easily seen with an ophthalmoscope. For most patients it is a benign disorder not associated with neurological disease. Microsaccadic flutter as opposed to typical ocular flutter and opsoclonus may have a different underlying mechanism; disruption of pause cells *and* saccadic pulse generator interconnections may cause opsoclonus and flutter while microsaccadic flutter and voluntary nystagmus may be due to malfunction of pause cells alone.

ACKNOWLEDGEMENTS

This research was supported by grants R01-EY05505 (T.C.H.) and R01-EY01849 (D.S.Z.) from the National Institutes of Health and a fellowship in neurosciences from the Charles A. Dana foundation awarded to J.A.

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(*Received August 22, 1989. Revised February 14, 1990. Accepted April 3, 1990*)

Brain (1991), **114**, 473–495

DEVELOPMENT OF LANGUAGE IN SIX HEMISPHERECTOMIZED PATIENTS

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SUMMARY

The development of language skills in the isolated right hemisphere was investigated by comparing the performance of 3 left hemispherectomized with that of the 3 right hemispherectomized patients and three groups of control subjects on baseline clinical measures of language and on production and judgement of morphological markers. The initial brain insult in the hemispherectomy patients of each group had occurred either during early, middle or late childhood. The left hemispherectomy patients were severely impaired in language processing across all three stages of language development. The consequences of a right hemisphere insult on language development were more restricted, with deleterious effects being apparent only in the case in which the lesion was acquired during early childhood.

INTRODUCTION

The most valid measures of the right cerebral hemisphere's capacity for language are those obtained when right-sided processing is known to be independent of left-sided input. An opportunity to obtain such measures is provided by the study of a small number of patients who have undergone either hemidecortication or callosal section for the treatment of intractable epilepsy. Based on the study of such patients, during the past 15 yrs, there have been diverse reports highlighting the capacity of the isolated right hemisphere for general language ability and also its restrictions and relative incapacity for selective aspects of linguistic processing (Dennis and Whitaker, 1976; Zaidel, 1980). In general, studies of split brain patients have concluded that the right hemisphere has a basic visual and auditory lexicon consisting of concrete and high-frequency words. Further, the disconnected right hemisphere can recognize and comprehend spoken or written words from this lexicon, even though it cannot demonstrate its comprehension through speech. In contrast to these domains of ability, the right hemisphere has difficulty in comprehending abstract, low-frequency and low-imagery words, manipulating subtleties of grammatical structure and analysing words according to their phonetic features (Zaidel, 1980; Gazzaniga, 1983).

Studies of patients who have undergone hemidecortication have concentrated on specifying the subtle deficits of the right cerebral hemisphere in the processing of selective aspects of language comprehension. The general conclusion of these reports is that despite

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the early onset of cerebral insult leading to right hemisphere mediation of speech and language functions, residual deficits in syntactic comprehension prevail (Dennis and Whitaker, 1976). Specifically, left hemidecorticated patients are reported to be impaired in the comprehension of passive negative constructions (e.g., the boy was not hit by the girl). For these sentences to be interpreted correctly, the subject-verb-object (SVO) sequence must be disregarded and replaced by a decoding strategy which reverses the order of mention of the SVO relationship. Accurate comprehension of such sentences thus requires syntactic rather than pragmatic knowledge. Dennis and Kohn (1975) reported that patients after left hemispherectomy are unable to adopt a reversal strategy and, as a result, misinterpret negative passive sentences.

Dennis and her colleagues (Dennis *et al.*, 1981; Lovett *et al.*, 1986) have addressed the question of reading, writing and language production in patients with hemispherectomy, and they have reported additional examples of the limitations of the isolated right hemisphere in language processing. On the whole, these data have been interpreted as evidence against an equipotentiality view of the two cerebral hemispheres.

Recently, this group of studies and the conclusions derived from them have come under sharp criticism from Bishop (1983, 1988). He maintains that since the experimental tasks administered to hemidecorticated patients were not validated on neurologically intact control groups, the patients' apparent deficit may be related to the large variance that they demonstrated, although no explanation has been provided for the occurrence of the variance itself. Indeed, when the factors of age and low IQ are allowed to range widely, neurologically intact control subjects also demonstrate large variance in task performance. Bishop's criticisms cast doubts on the anti-equipotentiality position and highlight the need for rigorous standards with such a select group of patients.

The equipotentiality view of the two hemispheres is supported by reported findings that neither left nor right hemidecortication performed subsequent to early acquired cerebral insult results in persisting speech or comprehension disorders, although initial injury to the left and sometimes the right cerebral hemisphere may cause transient dysphasia (McFie, 1961; Basser, 1962). Such data have traditionally been considered as evidence in support of the notion of 'plasticity' of the immature central nervous system. It should be pointed out, however, that incidence of 'speech and comprehension disorders' has usually been assessed in purely clinical terms and to date there are no conclusive investigations of 'higher order' qualitative differences between the productive and receptive language abilities of left and right hemidecorticated patients. When such investigations have been reported, methodological considerations have called into question the data and therefore the conclusions derived from them.

For example, Ogden (1988) has reported long-term follow-up data on language and memory skills of 2 patients who underwent left hemispherectomy during adolescence consequent upon initial injuries acquired during early childhood. In the absence of data on patients with right hemispherectomy, Ogden compared her findings with those reported by Dennis and Kohn (1975) and with normal standards, concluding that the data supported the equipotentiality view.

Unfortunately, interest in the equipotentiality issue has resulted in the neglect of a question that may be of greater theoretical and practical importance, namely, the contribution of the right hemisphere to the development of language functions. On the basis of existing clinical and experimental evidence (e.g., Basser, 1962), it appears that the right cerebral hemisphere is perfectly capable of mediating at least gross processing of receptive and productive language functions provided the injury has been sustained during early childhood. What is not clear is whether the right hemisphere makes a qualitatively different contribution to higher order processing than the left and, if so, whether the nature of this contribution varies as a function of the stage of language development at the time of the initial insult.

The present investigation was undertaken to help resolve some of the foregoing issues. The first objective was to examine possible qualitative differences between left and right hemidecorticated patients in the processing of morphological structure. The second was to determine whether or not the degree and the quality of any hemisphere differences were related to the stage of language development and to the age at which the initial hemisphere injury was acquired.

MATERIAL AND METHODS

Subjects

Six patients (3 left; 3 right) who had undergone hemispherectomy (Adams' modification; Adams, 1983, *see* Appendix 1) for relief from intractable epilepsy were studied in pairs formed on the basis of age at the commencement of epilepsy (early childhood; middle childhood, late childhood). Four patients underwent total, while 2 underwent subtotal removals (*see* Appendix 1). When appropriate, comparisons were made between the performance of pairs of patients to highlight the effects of the variable of age at injury.

The hemispherectomy patients were divided into two groups on the basis of side of hemisphere removal. Comparisons between the two hemispherectomy groups revealed the effects of laterality of lesion. Performance of the two hemispherectomy groups was in turn compared with a group of normal subjects, a group of patients who had suffered from long-standing focal epilepsy and had undergone unilateral left temporal lobectomy during adolescence, and a group of patients with congenital right-sided hemiplegia without accompanying seizures. Throughout, the most critical group comparisons were between the two hemispherectomy groups. Comparisons with the patient and the normal control groups only served to place in a broader perspective the performance levels of the hemispherectomy groups.

Early childhood damage—left hemisphere removed (ECD, L-X). Case 1 (S.F.) was born following a full-term pregnancy and delivery. The family history was unremarkable and she developed normally, achieving appropriate milestones. When she was 15 months old, she suffered a 1 h seizure in association with fever. She had residual twitching in her right limbs and, after 10 days, a measles rash appeared. A right hemiparesis was noted following this acute illness and shortly thereafter seizures commenced. These consisted of a cephalic aura and stiffening of the right limbs. Seizures typically lasted 1–2 min, but occasionally they became generalized. S.F. started normal schooling at the age of 5 yrs, but by the age of 7 yrs she had fallen behind her peers and was transferred to a special school.

When examined preoperatively, she had a right infantile hemiplegia with appropriate sensory loss and a homonymous hemianopia. An EEG showed left frontal abnormalities. A CT scan showed extensive left porencephaly with preservation of some frontal cortex. Preoperative psychometry revealed scaled scores of (5) and (2) on Vocabulary and Block Design subtests of the WISC-R.

When aged 16 yrs, S.F. underwent a left total hemispherectomy. Postoperative progress was good. Within the first year of operation there were occasional myoclonic jerks but otherwise there were no further seizures. Intellectual functioning remained unaltered relative to preoperative levels. The pathology report indicated a shrunken left cerebral hemisphere weighing 180 g (normal weight range 500–650 g). There were numerous features suggestive of a 'circulatory' disturbance and no evidence of the residua of measles encephalitis.

Early childhood damage—right hemisphere removed (ECD, R-X). Case 2 (M.S.) was one of identical twins born following a breech delivery. She developed normally, achieving appropriate milestones. At the age of 2 yrs, she was noticed to be crying and unable to stand. She had a left hemiplegia which had developed overnight. When she was aged 3 yrs, regular seizures began. She would say 'mum, mum', become vacant, shake and fall. Within months, she developed other kinds of seizures including drop attacks, episodes of serial seizures and attacks beginning with deviation of the head and eyes to the right and shaking of the left limbs, progressing to a generalized convulsion.

When examined preoperatively, M.S. had an infantile left hemiplegia without a visual field defect. A CT scan showed loss of cerebral cortex on the right consistent with right middle cerebral artery ischaemia. An EEG carried out a year before operation indicated mainly right-sided slow waves with occasional bilateral spikes; the preoperative EEG showed bilateral abnormalities. Preoperative psychometry revealed a verbal IQ of 55 and a performance IQ of 73. She underwent a total right hemispherectomy when she was aged 26 yrs. Postoperatively, she had a left homonymous hemianopia. Regular seizures were controlled, but two episodes of status occurred. Histopathological examination indicated extensive scarring of the right hemisphere which weighed 300 g.

Middle childhood damage—left hemisphere removed (MCD, L-X). Case 3 (S.S.) was born following an induced labour and breech delivery. Early development and milestones were entirely normal and there were no serious childhood illnesses. There was no family history of epilepsy. When he was aged 4½ yrs, he had an episode during which he suddenly became aphasic, vomitted and then had a right-sided convulsion. Two further episodes involving generalized convulsions occurred over the next 4 months. Within 2 yrs, the pattern of seizures became well established and involved right-sided focal motor seizures lasting 2–3 min. The frequency of these attacks increased in severity such that by the age of 8 yrs he was having up to 5 focal motor seizures per hour. Thirty months after the first episode, S.S. was admitted to hospital with epilepsia partialis continuans and a right hemiparesis.

CT scans carried out between the ages of 6 and 8 yrs showed progressive left cerebral atrophy Similarly, EEG recordings carried out during the same period indicated only left-sided abnormalities, but by the time he was aged 8½ yrs, some right frontal abnormalities were also apparent. During a 2 yr period, intellectual ability decreased by 30 points, and, preoperatively, his verbal IQ was 74 and performance IQ was 60.

A subtotal left hemispherectomy was carried out when he was aged 8¾ yrs. Postoperatively, recovery was good and seizures were totally arrested. Eight years following the operation, S.S. was completely weaned off his anticonvulsant regime. Histopathology indicated that the entire excized neocortex, weighing 260 g, was affected by extensive nerve loss, fibrillarly astrocytosis with microglial nodules and perivascular cuffing by lymphocytes and macrophages. On the basis of the medical history and histopathology, the diagnosis of Rasmussen's encephalitis (Rasmussen, 1978, 1983) was made.

Middle childhood damage—right hemisphere removed (MCD, R-X). Case 4 (L.W.) was the product of a full-term normal pregnancy and delivery. Developmental milestones were normal and the family history was unremarkable. At the age of 5 yrs L.W. developed erythema nodosum related to a primary tuberculous lesion, and received antituberculous treatment for 6 months. When almost 6 yrs old, she had a grand mal seizure when pyrexial. Within months, left-sided focal seizures developed and continued until the age of 8 yrs when a full investigative screen was found to be negative, but weakness of the left limbs was noted. By the age of 9 yrs, she had a persistent left hemiparesis and was having about 15 attacks per day. Shortly thereafter, she went into refractory left-sided focal motor status. On recovery, there was bilateral leg weakness (left > right) and she was doubly incontinent. Following this episode, the frequency of the seizures varied from 5 to 20 per day.

Preoperatively at the age of 10 yrs, L.W. was alert with full visual fields. She had a left hemiparesis, clonus at the left ankle and a left extensor plantar response. Pyramidal weakness of the right leg with increased tone was also noted. CT scans performed when she was aged 7–10 yrs old indicated right-sided cerebral atrophy, chiefly within the frontal and sylvian regions. EEG during the same period showed mixed irregular slow and spike activity on the right, mainly in the frontocentrotemporal regions. Psychometry revealed a verbal IQ of 92 and a performance IQ of 69.

Following a right subtotal hemispherectomy L.W. became seizure-free and has remained so to date. She is still continuing on a moderate dose of anticonvulsant therapy. Her walking has improved such that she can move around with a stick, and her incontinence ceased within 2 yrs after the operation. Histopathology indicated a specimen weighing 210 g with patchy destruction throughout and focal inflammatory changes. The diagnosis was Rasmussen's encephalitis.

Late childhood damage—left hemisphere removed (LCD, L-X). Until the onset of her illness at age 13 yrs, Case 5 (N.I.) was a normal right-handed girl, attending secondary school with a performance in the average range, and with no personal or family history of neurological abnormality. At this age, she suddenly presented with a generalized convulsion preceded by lethargy and episodes of dysphasia for 6 weeks. Over the next 4 months she had further generalized attacks, 1 of these followed by transient weakness of the right limbs.

At 8 months after the first generalized convulsion, focal motor attacks involving the right arm and leg appeared, and within weeks these became epilepsia partialis continuans. Attacks lasted 30–40 s and were generally preceded by an unpleasant taste in the mouth. On examination 1 yr after the onset of the illness, N.I. had a right hemiparesis, an extensor right plantar response, a right homonymous hemianopia and some dyspraxia. She had become left-handed and was sufficiently disabled that she had to hold on to furniture to move around.

From the outset the attacks included dysphasia. Although speech and language functions were frequently interfered with as a result of the seizures and postictal states, her speech deteriorated radically only some 14 months after the onset of her illness.

- Diagnostic tests had consistently implicated her left hemisphere. EEG showed widespread left hemisphere abnormalities. Although CT scans initially did not indicate any gross changes, within 18 months postonset, they indicated left hemisphere atrophy. The preoperative scan showed moderate left hemiatrophy with an area of low density in the left posterior region of apparent ischaemic origin.

Preoperative psychometry showed scaled scores of (1) on both the vocabulary and comprehension subtests and scaled scores of (4) and (1) on block design and object assembly subtests of the WISC-R.

A sodium amytal test (Branch *et al.*, 1964) was performed preoperatively to determine whether her right hemisphere was capable of subserving gross speech and memory functions. Injections were given on 2 separate days, and the left hemisphere was injected three times, the right hemisphere once. The evidence derived from the left-sided injections was inconclusive as she repeatedly fell asleep, but speech arrest coincident with the onset of hemiparesis was evident only when the right cerebral hemisphere was inactivated.

Before operation, N.I. was having continuous seizures. She had a right facial palsy, a 'cortical' weakness of the right upper limb with no useful movement in the hand, and similar weakness of her right lower limb. She had 'cortical' sensory loss involving the whole right side of the body, and a complete right homonymous hemianopia. There were increased tendon jerks and an extensor plantar response on the right side. Her speech showed a moderate expressive dysphasia

A total left hemispherectomy was undertaken 30 months after the onset of her illness and when she was aged 15 yrs. Histopathology showed widespread abnormalities affecting the left neocortex. Major structural landmarks were either shrunken because of neuronal cell loss or grossly misrepresented as a result of the disease process. The postoperative diagnosis was Rasmussen's encephalitis.

Seizures were totally arrested following the operation and she has remained seizure-free to date. She is still on a moderate anticonvulsant regime. Her neurological state has remained unchanged.

Late childhood damage—right hemisphere removed (LCD, R-X). Case 6 (H.P.) was the product of a normal pregnancy and delivery. Shortly after her birth, she was diagnosed as suffering from Turner's syndrome. This chromosomal abnormality is reported to be associated with an intellectual deficit (Money, 1964; Buckley, 1971), although more recent reports indicate that intelligence is sometimes spared (Menkes, 1980); indeed this child went on to develop normally and to achieve appropriate milestones. She was right handed, and her early years were medically unremarkable. She attended a normal school locally and was performing in the above average range in all academic subjects until her epilepsy commenced. When H.P. was nearly 10 yrs old, she was found totally unresponsive, lying on her left side with her eyes closed. A month later, a similar episode occurred, and over the ensuing months a pattern of minor seizures changed to focal attacks involving the left limbs. The severity of these increased such that within months she was admitted to hospital with epilepsia partialis continuans and a moderate degree of Todd's paralysis.

A CT scan carried out at this stage indicated some degree of right cerebral atrophy. EEG showed multifocal discharges maximal in the right rolandic region. A sleep EEG showed continuous epileptic discharges over the right hemisphere accompanied by movements of the left arm and leg. Neurological examination revealed increased tone on the left side with decreased power and coordination. Major neurosurgery was contemplated at this stage but the decision was postponed in favour of drug therapy Over the ensuing 5 yrs, many attempts were made to bring the seizures under control using different regimes of anticonvulsant medications and corticosteroids, but there was no enduring success.

Preoperatively, H.P. was having frequent seizures, up to 6 per hour, involving the left arm and leg with deviation of the head to the left. Neurological examination indicated an extensive right hemisphere lesion. She had 'cortical' weakness of the left upper limb with no useful movement in the hand, and a similar weakness of her left leg. She had a left homonymous hemianopia, and a left hemisensory loss with impaired joint position sense. There were increased tendon jerks down the left side with a left extensor plantar response. Preoperative CT scanning had shown increasing right hemiatrophy. EEG revealed gross abnormality with runs of high voltage sharp-slow wave complexes and no normal background activity. Psychometry before operation showed a verbal IQ of 82 and a performance IQ of 67. Verbal memory was mildly impaired while spatial memory skills were severely deficient.

A total right hemispherectomy was carried out when H.P. was aged 15 yrs and, on the basis of medical history and histopathological features, the diagnosis of Rasmussen's encephalitis was made. Although macroscopic examination revealed a curious absence of focal lesions, detailed histology of the cortex showed varied and extensive degrees of damage and atrophy. In the most severely affected areas, there was narrowing of the full thickness of the cortex with neuronal loss, and astrocytic and vascular proliferation.

Following operation H.P.'s seizures were totally arrested, and she was weaned off anticonvulsant medication within the same year.

The clinical histories for Cases 5 and 6 have also been documented as part of another investigation (Patterson *et al.*, 1989).

Hemispherectomy groups. The hemispherectomy patients were divided into two groups on the basis of the side of hemisphere removal (left hemisphere removed, L-X; right hemisphere removed, R-X). Mean age at onset of seizures for the (L-X) and (R-X) groups was 6.3 and 6.0 years, respectively Mean age at testing was 17.9 (SD = 0.7) for the L-X group and 20.4 (SD = 5.3) years for the R-X group.

Control groups. Two patient groups and a group of normal children served as controls. Group (LT) consisted of patients with left temporal lobectomies (n = 8). These patients had all had focal epilepsy dating back to early childhood and had undergone elective neurosurgery during adolescence. The earliest age at onset of seizures was 24 months and the latest 11.8 yrs of age (mean 5.9 yrs). Mean age at testing was 18.7 yrs (SD 2.6). In the majority of cases the aetiology was mesial temporal sclerosis. In addition, there were 2 cases of indolent tumours and 1 case of nonspecific necrosis. The temporal lobectomies were standard en bloc removals from the anterior pole of the temporal lobe, along the sylvian fissure ranging from 5.0 to 6.5 cm along the base of the brain. The average extent of removal along the midtemporal gyrus of the adult was 6.0 cm and a proportionate amount in children as judged by the length of the temporal lobe. The superior temporal convolution was always spared. The temporal lobe excisions included variable amounts of the dorsolateral part of the amygdala (1.0–3.0 cm) and the hippocampus (0.5–5.5 cm). On the basis of preoperative sodium amytal tests, patients with bilateral or right hemisphere mediation of speech and language functions were excluded from the group. Group (LT) was selected as a control group because it shared with the hemispherectomy groups the long-standing history of seizure disorder dating back to early childhood and leading to surgical intervention during adolescence.

Group (LC) consisted of patients who had had a unilateral and nonprogressive cerebral insult congenitally or perinatally (n = 10). While all patients had moderate to severe hemiplegia, none had any history of seizure disorder. Mean age at testing was 13.3 yrs (SD 3.2). Although sodium amytal test results were not available, it is suggested that in view of early left hemisphere injury affecting the central strip and demonstrations of strong sinistrality, speech and language functions may be mediated by the right cerebral hemisphere in at least some members of this control group (Vargha-Khadem *et al.*, 1985). Group (LC) was selected as a control group because it shared with the hemispherectomy groups the history of a cerebral lesion resulting in a clinically verified hemiplegia.

Group (NC) consisted of 12 normal children who were selected from inner and outer London area schools. While control groups (LT) and (LC) participated in all tasks which were administered to the hemispherectomy patients, group (NC) served to provide normative standards for the two experimental tasks and for the baseline language tests. Subjects in group (NC) were aged 13.2–15.6 (mean 14.4) yrs. Four children (2 males, 2 females) were tested at each of the age levels.

Material and procedures

In order to provide indications of cerebral language representation, and to establish baseline levels of intellectual, memory and language functioning, all patients were administered the following tests.

Handedness inventory. To quantify hand preference, an adaptation of the handedness questionnaire of Crovitz and Zener (1962) was administered. Hand preference was estimated by asking the subjects to indicate which hand they normally used to perform 18 common actions, some of which were bimanual. The scale ranged from 18 to 90. Scores below 30 were considered indicative of strong right-handed tendencies and scores above 55 suggested strong left hand preference (Milner, 1975).

Intelligence. One of the Wechsler Intelligence Tests (WISC-R; WAIS; WAIS-R) was administered depending on the age and previous testing experience of each patient.

Memory. The Wechsler Memory Scale was used to obtain a memory quotient (MQ). For patients who were below the age of 12 yrs, the Taylor children's stories (Milner, 1975) replaced the adult stories constituting the Logical Memory subtest. Additional age corrections were made in the calculation of the MQ to accommodate the relatively younger ages of the patients.

Object naming. The Oldfield and Wingfield (1965) picture naming test, consisting of 36 line drawings of objects, was administered. Accuracy and reaction times for naming to confrontation were recorded.

Language comprehension. The Token Test of Auditory Language Comprehension (De Renzi and Vignolo, 1962) was used to assess the subjects' ability to arrange tokens of various shapes, colours and sizes according to spoken instructions.

Receptive grammar. The Test for Reception of Grammar (Bishop, 1982) evaluated the subject's comprehension of various grammatical structures, such as singular/plural personal pronoun, reversible active, singular/plural noun inflection, comparative/absolute, reversible passive, etc. The subject selected 1 out of 4 pictures corresponding to a spoken sentence. The test consisted of 20 blocks, and the criterion of 'passing' a block was correct picture matching on all 4 items within the block. In view of the controversy surrounding the issue of deficits in comprehension of syntax by left hemidecorticated patients, particular attention was paid to the analysis of TROG error scores.

Production and reception of grammar were further investigated through experimental tasks designed to assess knowledge of morphological markers.

Production of morphological markers. Several modifications have been made (Berry, 1977; Palmer, 1983; R. W. Barron, S. M. Palmer and M. W. Lovett, unpublished observations) to elaborate the original test of Berko (1958) which employed the use of nonsense words to tap knowledge of English morphology. Thus young children were read sentences which were accompanied by pictures and then expected to complete the sentences (e.g., 'yesterday, I saw a nad. Then I saw another one. Yesterday, I saw two .....' The correct response would be 'nads'). The reliability of this type of task in eliciting appropriate morphological markers is reportedly high (Vogel, 1975).

While this experiment was based on Berko's original idea, it made use of the modifications introduced by Berry-Talbott and Palmer and further extended the design to accommodate the linguistic standards of the patients under study. To ensure that subjects were capable of producing the appropriate morphological markers using English words, they were pretested on the control version of the experimental task.

It is generally accepted that morphological markers may be divided into two subgroups, each with somewhat different characteristics (Lyons, 1968; Tomori, 1977; Dever and Gardner, 1970). Thus inflectional morphemes preserve the original class of the word since their form dictates their relationship to other words in a sentence (e.g., the sun *is* shin*ing*). In contrast, derivational morphemes change the class of the original word and in so doing, provide semantic information as well (e.g., the plane landed gent*ly*).

For the control task, 20 English sentences consisting of 10 inflectional and 10 derivational morphemes were used. Sentences involving inflectional markers consisted of the following forms: the third person singular verb (she swim*s*), the past tense (e.g., he talk*ed*), the present participle (e.g , bark*ing*), the comparative (e.g., small*er*) and the superlative (e.g., tall*est*). The sentences involving derivational markers included the adverbial suffix *ly* (e.g., happily), the adjectival suffix *y* (e.g., hairy), the agentive noun suffix *er* (e.g., runner), the adjectival suffix *less* meaning devoid of (e.g., sleeveless) and the gerund *ing* (e.g., as easy as swimming). For all sentences, the test item was always the last word in the sentence.

The experimental task was identical to the control task in every respect except that in each of the 20 sentences 2 nonsense words were used, 1 to represent the agent and the other, the action or state of the agent, i.e., the target word which must be inflected (e.g., this nad can tope. What is he doing? He is .....toping).

Forty line drawings were designed, one to correspond to each of the sentences. In the case of the experimental task where nonsense words were used, drawings of fictitious creatures were used to convey actions or states of being. Fig. 1 illustrates exemplars of the drawings and the accompanying sentences for the control (left) and the experimental (right) tasks.

Subjects were requested to look at each drawing and help complete the sentence read by the experimenter. The sentence was repeated under the following circumstances: (1) if the subject offered no response within 15 s; (2) if the subject claimed that he or she did not know the answer; (3) if the subject requested repetition because he or she had forgotten the sentences; (4) if the subject produced an English word instead of a nonsense word in the experimental condition; (5) if the subject produced a syntactically correct nonsense word phrase instead of the correctly inflected nonsense word (e.g., 'was tading' instead of 'taded'). In

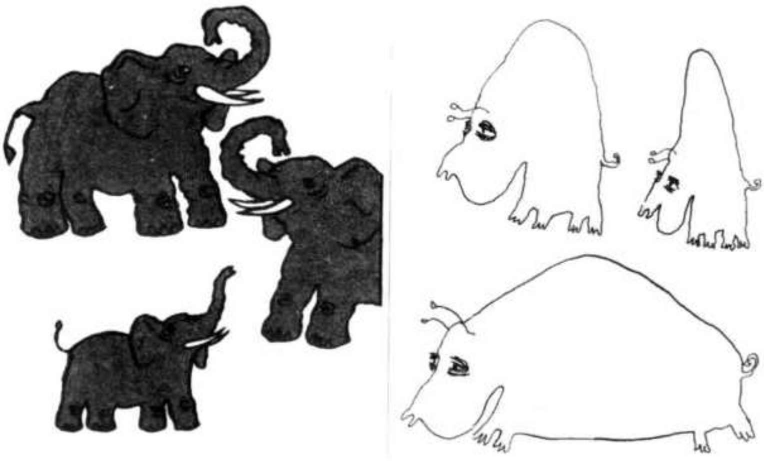

FIG. 1. Drawings accompanying the words (*left*) and nonwords (*right*) sections of the morphological production task.

general, the control task provided the subjects with the necessary experience to perform appropriately and, as a result, they had no difficulties with the manipulation of nonsense words in the experimental task. Each task was scored out of 20.

Judgement of morphological markers. This task was not designed as an analogue of the production version, but rather as a means of assessing receptive sensitivity to syntactical constraints within sentences. Although subjects were not asked to demonstrate comprehension of sentences read to them, they were required to make accuracy judgements on appropriate and inappropriate morphological markers. Once again a control task involving English words was first constructed. Twenty-four pairs of sentences were prepared with 12 pairs consisting of sentences wherein the target word was appropriately inflected in one, but not the other (e.g., the boy was sing*s* a tune; the boy was sing*ing* a tune). In order to reduce chance performance and to encourage careful scrutiny of the sentences, 12 trials wherein neither alternative was correct (e.g., The boy was sing a tune; the boy was sing*ly* a tune) were randomly mixed with the other 12 trials. Since the focus of this task was on receptive sensitivity to syntactical constraints within sentences, morphological markers were mainly selected from the inflectional category. Two exemplars were used for each of the 5 different inflectional morpheme items (i.e., the present tense *-es* or *-s*; the past tense *-ed*; the present participle *-ing*; the comparative *-er*; the superlative *-est*). In addition, 2 exemplars of a derivational morpheme (the adverbial suffix *-ly*) were also included.

Although pairs of sentences were printed on 5×7 inch cards and placed in front of the subject, the experimenter always read aloud the sentences regardless of the reading proficiency of the subjects. To alleviate the burden on short-term verbal memory, sentences were reread to the subjects on request. Subjects were required to indicate whether sentence 1 or 2 or neither was correct. Before the commencement of the task, subjects received 2 pretest trials providing exemplars of the 2 types of sentences. If subjects had difficulties with the pretest trials, other examples were provided until the experimenter was assured that task requirements were fully understood before testing was begun.

The experimental version was identical to the control task except that in each sentence pair there were 2 nonsense words. On half the trials, 1 of the nonsense words was appropriately inflected (e.g., planes are donk*er* than trains; planes are donk*est* than trains). On trials wherein both alternatives were incorrect, neither nonsense word was appropriately inflected (e.g., planes are donk than trains; planes are donk*ing* than trains).

Group (NC) provided normative standards against which the performance of the hemispherectomy patients was evaluated.

RESULTS

Data derived from hemispherectomy patients were treated primarily as group data because this provided the opportunity to examine the overall effects of the side of hemisphere removal collapsed across the three stages of development. In view of the small number of patients in each of these two groups (L-X and R-X), direct comparisons between them, which were the most critical comparisons for the purpose of this study, were carried out using nonparametric statistics (Mann-Whitney, Siegel, 1956). To enable direct comparisons between these two groups and the three control groups, however, parametric statistics (analyses of variance and covariance) were used. The Newman-Keuls multiple-comparisons method was adopted for all a posteriori testing (Winer, 1962). To ensure that variations in age and IQ did not contribute to possible differences between groups on the language tests, analyses of covariance, partialling out the effects of age and IQ, were performed on the scores derived from all patients groups. Intelligence quotients were not available for the (NC) group and, for this reason, separate analyses of covariance partialling out the effects of age, were performed on all the measures derived from the five groups. Since the results of both sets of analyses were concordant, the findings from the latter set are reported, as these include the performance of the (NC) group.

Wherever appropriate, individual hemispherectomy patients' scores were compared with those of the three control groups, with impairments being defined as mean scores falling 2 or more SDs below the relevant control means. Finally, case by case comparisons were made between left and right hemispherectomy cases at various ages at injury to examine the influence of this variable.

Baseline measures

Tables 1 and 2 present the relevant data for the hemispherectomy cases as well as the means and SDs for the four patient groups. Preoperative (when available) and postoperative intelligence subtest scores for the hemispherectomy patients are provided in Appendix 3. Mean postoperative intelligence subtest scores for the patient control groups are provided in Appendix 4.

TABLE 1. AGE, HANDEDNESS, MEMORY QUOTIENT AND WECHSLER INTELLIGENCE RATINGS FOR HEMISPHERECTOMY CASES

Case	*Age at testing (yrs:mos)*	*Handedness (18–90)***	*MQ*	*Verbal IQ*	*Performance IQ*
1 (ECD, L-X)	18 5	90	66	69	63
3 (MCD, L-X)	17:0	90	61	69	70
5 (LCD, L-X)	18·0	90	62	55	80
2 (ECD, R-X)	26·5	18	56	55	61
4 (MCD, R-X)	16:8	18	62	68	49
6 (LCD, R-X)	18 0	18	83	80	75

** Scale of Crovitz and Zener (1962)

Examination of the hemispherectomy data showed that except for the (LCD, L-X)

TABLE 2 AGE, HANDEDNESS, MEMORY QUOTIENT AND WECHSLER INTELLIGENCE RATINGS FOR PATIENT GROUPS. MEANS AND SDs*

Group	*Age at testing (yrs·mos)*	*Handedness (18–90)***	*MQ*	*Verbal IQ*	*Performance IQ*
L-X	17:9	90	63.0	64.3	71.0
(n=3)	(0.7)	(0.0)	(2.6)	(8 1)	(8.5)
R-X	20:4	18	67.0	67 7	61.7
(n=3)	(5.3)	(0.0)	(14 0)	(12.5)	(13.0)
LC	13 4	74 8	99 1	100.4	95.0
(n=10)	(3.2)	(26.9)	(7.0)	(12.6)	(15 1)
LT	18.8	34.7	89 7	89.5	93 6
(n=8)	(2.6)	(22.3)	(7.1)	(10 1)	(11.6)

* SDs in brackets. ** Scale of Crovitz and Zener (1962)

and (MCD, R-X) cases, there were no reliable VIQ-PIQ discrepancies consistent with the side of hemisphere removal. In all cases, the MQ, which predominantly reflects verbal memory processing, was consistent with the verbal IQ. Nonparametric tests revealed no significant differences between the (L-X) and (R-X) groups on the measures of verbal IQ ($U = 4.5$, $P = 1.0$), performance IQ ($U = 2$, $P = 0.27$) and MQ ($U = 4.5$, $P = 1.0$).

One-way ANOVA based on the four patient groups indicated significant main effects of age ($F = 5.5$, $P < 0.007$), handedness ($F = 9.91$, $P < 0.001$), MQ ($F = 24.41$, $P < 0.001$), Verbal IQ ($F = 11.49$, $P < 0.001$) and Performance IQ ($F = 7.03$, $P < 0.002$). Post hoc analyses showed that the (LC) group was significantly younger relative to all other groups. The (L-X) and (LC) groups had significantly higher handedness scores (indicating strong sinistrality) compared with the (LT) and (R-X) groups whose mean scores were consistent with ambidextrous and strong dextral tendencies, respectively. Lastly, the (LC) group had significantly higher MQ scores relative to all other groups, while the mean MQ of the (LT) group was significantly higher only than those of the (L-X) and (R-X) groups. For both intelligence quotients, the (LC) and (LT) groups obtained higher standards compared with the (L-X) and (R-X) groups. There were no other significant differences between groups.

A two-way analysis of variance (ANOVA) assessed differences between groups in relation to verbal/performance IQ discrepancies. Results confirmed those shown by the univariate analysis (main effect of groups, $F = 10.03$, $P < 0.0001$) but indicated no interaction of verbal IQ and performance IQ by group. There were no other significant differences indicated.

Baseline language measures

Various measures derived from the baseline language tests were subjected to nonparametric treatments for the (L-X) and (R-X) groups and one-way analyses of covariance (ANCOVA) (partialing out the effects of age) for the five subject groups. Means and SDs derived from the analyses of covariance (lower section) and the individual data from the hemispherectomy patients (upper section) are presented in Tables 3 and 4.

TABLE 3 BASELINE LANGUAGE MEASURES FOR HEMISPHERECTOMY CASES

	Object naming test		*Test for Reception of Grammar (TROG)*			*Token*
Case	*Accuracy (%)*	*RT (s)*	*Blocks passed (/20)*	*Errors (/80)*	*1st error (1–80)*	*Test (%)*
1 (ECD, L-X)	63.8	3.4	13	15	47	72.6
3 (MCD, L-X)	63.8	2.7	10	20	36	74.0
5 (LCD, L-X)	38.8	5.2	12	20	29	72.6
2 (ECD, R-X)	67.0	3.6	15	11	49	63.0
4 (MCD, R-X)	78.0	1.9	17	4	39	91.9
6 (LCD, R-X)	88.8	1.6	17	3	59	95.1

TABLE 4 PERFORMANCE ON BASELINE LANGUAGE MEASURES FOR SUBJECT GROUPS MEANS* AND SDs**

	Object naming test		*Test for Reception of Grammar (TROG)*			*Token*
Groups	*Accuracy (%)*	*RT (s)*	*Blocks passed (/20)*	*Errors (/80)*	*1st error (1–80)*	*Test (%)*
L-X	55.3	3.7	12	18.3	37	73.2
	(14.4)	(1.3)	(1.5)	(2.8)	(9.1)	(0.8)
R-X	76.1	2.2	16	6.1	49	85.2
	(10.9)	(1.1)	(1.1)	(4.4)	(10.0)	(17.6)
LC	81.1	2.0	19	1.9	61	91.6
	(11.4)	(0.6)	(1.3)	(1.7)	(16.3)	(3.4)
LT	78.2	1.8	17	4.2	46	93.9
	(9.5)	(0.7)	(2.6)	(3.8)	(16.0)	(8.6)
NC	84.1	2.0	18	2.6	65	95.1
	(6.7)	(0.5)	(1.4)	(2.1)	(11.6)	(2.1)

* All means are corrected for age. ** In brackets.

Object naming accuracy. Mean object naming accuracy of the (R-X) group was significantly higher than that of the (L-X) group (U = 0.0, $P = 0.0495$). Similarly, the ANCOVA results indicated that the (L-X) group was impaired relative to all other groups (F = 4.99, $P < 0.003$) with no significant differences apparent between the remaining four groups.

Considered individually, the variable of age at injury differentially affected the left and right hemispherectomy cases. Thus for the right hemispherectomy patients, naming accuracy increased the later in life the initial insult occurred; in contrast, for the left hemispherectomy patients, naming accuracy was most severely affected when the injury was sustained during late childhood.

Reaction times to naming. Despite the fact that the reaction times of the (L-X) group were substantially longer than those of the (R-X) group, the difference was not statistically significant (U = 2.0, $P = 0.2752$). The longer reaction times of the (L-X) group were mainly created by the naming difficulties of the (LCD, L-X) case.

When reaction times of the five groups were compared by ANCOVA, however, the

(L-X) group was found to have significantly larger values relative to the other groups studied ($F = 4.77$, $P < 0.004$). Once again, the differences between the remaining subject groups did not reach conventional levels of significance.

TROG–blocks passed. The (R-X) group's performance was significantly better than that of the (L-X) group ($U = 0.0$, $P = 0.046$). Consistent with this finding, the test of covariance showed a significant main effect of groups ($F = 4.39$, $P < 0.01$). Post hoc comparison of means showed that the (L-X) group was impaired relative to all other groups; the (R-X) group, however, also showed significantly lower accuracy than the (LC) group ($q = 3.87$, $P < 0.05$). This difference was mainly attributable to the performance of the (ECD, R-X) case which was more than 2 SDs below the means of the (LC) and (NC) groups.

TROG–errors. Analyses indicated significantly more errors for the (L-X) than the (R-X) group ($U = 0.0$, $P < 0.046$) and a significant main effect of groups ($F = 20.42$, $P < 0.001$). Once again, post hoc comparisons showed that the (L-X) group's error score was significantly higher compared with all other group scores.

Further examination of patterns of errors indicated that all 3 left hemispherectomy patients had difficulties in understanding passive structures. In contrast, Case 4 (MCD, R-X), who had made errors on the two blocks preceding and following the passive structures, nevertheless passed every passive exemplar without difficulty. Similarly, Cases 6 (LCD, R-X) and 2 (ECD, R-X) experienced no problems in the processing of the passive sentences. (*See also* Appendix 2.)

As before, the age of the hemispherectomy patients at injury reveals a differential trend, with insults acquired later in life selectively enhancing performance in the (R-X) group but impeding processing in the (L-X) group.

TROG–first error. Despite the fact that first errors appeared earliest in the (L-X) group, the difference with respect to the (R-X) group did not reach significance (Kruskal-Wallis $Z = 2.33$, $P = 0.1266$). Similarly, there were no significant differences when all five subject groups were contrasted on this measure (Kruskal-Wallis $Z = 6.670$, $P = 0.1544$).

Token test. The group mean of the (L-X) patients was lower than that of the (R-X) patients; variability among the 3 (R-X) cases, however, prevented a statistically significant difference ($U = 3.0$, $P = 0.5066$). Note that the lowest Token Test score of all was obtained by the (ECD, R-X) case who, together with all 3 members of the (L-X) group, showed performance levels more than 2 SDs below the mean for the (LT) group.

When comparisons were carried out between the five subject groups, ANCOVA revealed a significant effect of groups ($F = 7.95$, $P < 0.001$). Post hoc analyses once again showed that only the (L-X) group mean was significantly different from all other groups.

Experimental measures

Production of morphological markers. Accuracy scores were calculated for overall performance as well as for inflectional and derivational structures of both words and nonwords. Table 5 presents the relevant data for the 6 hemispherectomy cases and Table 6 the means and SDs derived from ANCOVA performed on the scores of all subject groups.

Words. As a group, neither the overall accuracy ($U = 2.0$, $P = 0.238$) nor the inflectional accuracy ($U = 3.0$, $P = 0.4867$) or the derivational accuracy ($U = 4.0$,

TABLE 5. PERFORMANCE OF THE HEMISPHERECTOMY CASES ON THE MORPHOLOGICAL PRODUCTION TASK

			Words		*Nonwords*	
Case	*Words Total accuracy (%)*	*Nonwords Total accuracy (%)*	*Inflectional accuracy (%)*	*Derivational accuracy (%)*	*Inflectional accuracy (%)*	*Derivational accuracy (%)*
1 (ECD, L-X)	75.0	35.0	100.0	50.0	70.0	0.0
3 (MCD, L-X)	85.0	45.0	90.0	80.0	50.0	40.0
5 (LCD, L-X)	75.0	45.0	70.0	80.0	40.0	50.0
2 (ECD, R-X)	75.0	60.0	80.0	70.0	70.0	50.0
4 (MCD, R-X)	90.0	90.0	100.0	80.0	100.0	80.0
6 (LCD, R-X)	90.0	75.0	100.0	80.0	80.0	70.0

TABLE 6. PERFORMANCE OF SUBJECT GROUPS ON THE MORPHOLOGICAL PRODUCTION TASK: MEANS* AND SDs**

			Words		*Nonwords*	
Groups	*Words Total accuracy (%)*	*Nonwords Total accuracy (%)*	*Inflectional accuracy (%)*	*Derivational accuracy (%)*	*Inflectional accuracy (%)*	*Derivational accuracy (%)*
L-X	78.2 (5.8)	41.6 (5.8)	86.7 (15.3)	69.8 (17.3)	53.2 (15.3)	29.8 (26.4)
R-X	83.9 (8.7)	74.1 (15.0)	93.7 (11.5)	75.1 (5.8)	81.9 (15.3)	64.1 (15.3)
LC	93.7 (9.8)	85.0 (12.4)	97.6 (4.2)	84.7 (12.5)	88.5 (9.5)	81.8 (19.7)
LT	94.2 (6.5)	84.3 (13.9)	100.0 (0.0)	85.1 (11.9)	90.2 (13.6)	71.9 (20.0)
NC	97.9 (3.9)	88.2 (8.7)	98.1 (3.9)	89.5 (3.9)	94.3 (9.8)	76.8 (7.9)

* All means are corrected for age. ** In brackets.

$P = 0.7963$) of the (L-X) patients were significantly impaired relative to the (R-X) group. While these results were generally consistent with those derived from ANCOVA performed on the scores of the five subject groups, further significant differences between groups also emerged. Thus on the measure of total accuracy, the main effect of groups was significant ($F = 5.37$, $P < 0.002$). Post hoc analyses indicated that the (L-X) group was impaired relative to all except the (R-X) group. Furthermore, the (R-X) group was also impaired but only in relation to the (NC) group ($q = 4.43$, $P < 0.05$).

ANCOVA performed on the inflectional scores revealed a significant effect of groups ($F = 3.20$, $P < 0.02$), while those of the derivational scores did not reach conventional levels of statistical significance ($F = 2.58$, $P = 0.058$). Post hoc analyses indicated that once again only the (L-X) group's performance was impaired compared with all except the (R-X) group.

When considered individually, the production scores for the (L-X) cases were not consistently influenced by the variable of age at injury. While overall accuracy and accuracy on inflectional items were lowest in the (LCD, L-X) case, her performance on the derivational items was substantially better than that of the (ECD, L-X) case. Much the same pattern of results was indicated when individual (L-X) patients were compared with their (R-X) counterparts. Whereas the (MCD, R-X) and (LCD, R-X) cases scored higher on the 3 measures derived from production of words, the performance of the (ECD, R-X) case was similar to that of the (LCD, L-X) case.

Although group means of the (R-X) patients were consistently higher than those of the (L-X) patients, their patterns of performance were very similar. In fact 2 patients in each group performed at ceiling on the inflectional items.

Nonwords. Overall accuracy of the (L-X) group was significantly deficient relative to the (R-X) group (U = 0.0, $P = 0.0463$). When accuracy for inflectional and derivational morphological markers were considered separately, however, the means in each case fell short of statistical significance (U = 0.5, $P = 0.0765$). It is possible that with increased number of trials, significant differences for the two types of markers would have emerged.

Three separate one-way ANCOVA for production of nonwords indicated significant effects of groups in each case (total accuracy: F = 9.96, $P < 0.001$; inflectional accuracy: F = 7.62, $P < 0.001$; derivational accuracy: F = 5.25, $P < 0.003$). Post hoc analyses indicated that the (L-X) group was significantly impaired relative to all other groups.

Considered as a group, the (R-X) patients performed within the same levels as the two patient control groups on all measures of nonword morphological production; in relation to the (NC) group, however, the (R-X) mean scores were almost 2 SDs lower. This difference was mainly created by the performance of the (ECD, R-X) case which was more than 2 SDs below the means of the (LC) and (NC) groups on all three measures and below that of the (LT) group on inflectional accuracy only.

Examination of individual overall accuracy scores within the (L-X) and (R-X) groups showed that lowest performance was exhibited by the 2 cases with earliest ages at injury. At least in the case of the (ECD,L-X) patient, this was due to an exceptionally poor performance on the derivational items where not a single marker was correctly produced.

Comparison of words vs nonwords. Three separate two-way ANCOVA (repeated measures) indicated significant main effects of group on overall accuracy of words vs nonwords (F = 7.50, $P < 0.002$), on the comparison of inflectional vs derivational markers for words (F = 3.58, $P < 0.033$) and on inflectional vs derivational markers for nonwords (F = 7.89, $P < 0.001$). In addition, the main effect of task was statistically significant in each analysis (overall accuracy of words vs nonwords: F = 60.70, $P < 0.001$; words, inflectional vs derivational: F = 21.8, $P < 0.001$; nonwords, inflectional vs derivational: F = 12.27, $P < 0.002$). Finally, the comparison of overall accuracy of words vs nonwords indicated a significant interaction of groups by task (F = 8.41, $P < 0.001$).

Post hoc tests carried out to examine group differences in each analysis showed that the (L-X) group was significantly impaired relative to all groups, except in the comparison between inflectional vs derivational word items, where no deficits were found in relation to the (R-X) group. Collapsed over groups, results of post hoc analyses showed that performance on words was superior to nonwords and that for both words and nonwords, accuracy on inflectional items was higher than on derivational items. The interaction of groups by task confirmed the group differences reported separately for the word and nonword sections. Fig. 2 illustrates this interaction. Post hoc analyses revealed that

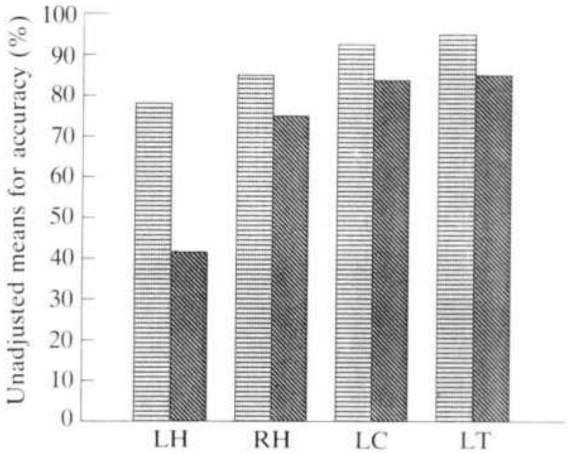

FIG. 2. Group by task interaction—morphological production of words and nonwords. Diagonal-hatched bars = nonwords. Cross-hatched bars = words.

once again, on morphological production of words, the (L-X) group was performing significantly below the standards of all groups except the (R-X) group, and below the standards of all patient groups on production of nonwords.

Judgement of morphological markers. Overall accuracy scores were calculated for both words and nonwords. Furthermore, to evaluate correct recognition of morphological markers independent of trials wherein both choices were incorrect, percentage scores were also calculated with such trials ($n = 12$) excluded from the overall score. Exclusion of such trials made both the control and the experimental versions of the task easier in that it provided the subjects the opportunity to judge accuracy of a morphological marker against an objectively correct alternative.

Words. Nonparametric comparisons of (L-X) and (R-X) group scores on the judgement task involving real words revealed no statistically significant differences (overall accuracy: $U = 2.0$, $P = 0.275$; trials without a correct alternative excluded: $U = 1.0$, $P = 0.104$). Table 7 presents the scores of the hemispherectomy cases on the morphological judgement task.

A separate one-way ANCOVA was carried out on the overall accuracy scores examining differences between the hemispherectomy groups and the (NC) group. The difference between group means was significant ($F = 10.97$, $P < 0.001$). Once again, on the basis of post hoc analyses, the (L-X) group was found to be impaired relative to both the (R-X) and the (NC) groups. Since it was felt that the impaired performance of the (L-X) group was compounded by the existence of trials wherein neither alternative was correct, a separate one-way ANCOVA was carried out with such trials excluded. Results of this analysis indicated that the difference between group means did not reach

TABLE 7. PERFORMANCE OF THE HEMISPHERECTOMY CASES ON THE MORPHOLOGICAL JUDGEMENT TASK

			Double negatives excluded	
Case	*Words Total accuracy (%)*	*Nonwords Total accuracy (%)*	*Words accuracy (%)*	*Nonwords accuracy (%)*
1 (ECD, L-X)	54.2	45.8	92 3	50 0
3 (MCD, L-X)	66.7	33 3	53.8	41.7
5 (LCD, L-X)	79 2	50.0	84.6	58.3
2 (ECD, R-X)	62.5	37.5	92.3	75.0
4 (MCD, R-X)	87 5	70 8	100.0	66.7
6 (LCD, R-X)	91.7	75.0	92 3	83.3

significance (F = 3.53, $P = 0.057$). Table 8 presents the group means for the morphological judgement task.

Examination of individual (L-X) patients' scores suggested that overall accuracy was most reduced in the (ECD, L-X) and least in the (LCD, L-X) case. This pattern failed to hold, however, when trials without a correct alternative were excluded.

TABLE 8. PERFORMANCE OF HEMISPHERECTOMY AND NORMAL CONTROL GROUPS ON THE MORPHOLOGICAL JUDGEMENT TASK MEANS* AND SDs**

			Double negatives excluded	
Groups	*Words Total accuracy (%)*	*Nonwords Total accuracy (%)*	*Words accuracy (%)*	*Nonwords accuracy (%)*
L-X	66.4 (12.5)	42.8 (8.7)	76.9 (20 3)	50.1 (8.3)
R-X	86.6 (15.8)	67 0 (20.5)	93.5 (4.4)	72.5 (8 3)
NC	86.5 (4.9)	64.1 (11.1)	92 9 (6 1)	83.6 (10.1)

* All means are corrected for age. ** In brackets

Analysis of individual (R-X) patients' scores indicated that for at least 2 of the 3 measures, accuracy scores were reduced most for the (ECD, R-X) patient. The scores of the (MCD, R-X) and (LCD, R-X) patients were considerably better and not substantially different from one another.

Nonwords. The overall accuracy of the (L-X) group on the nonword morphological judgement task was not significantly impaired relative to that of the (R-X) group (U = 2.0, $P = 0.275$). When trials without a correct alternative were excluded, however, the difference between the two groups became significant (U = 0, $P = 0.0495$).

Results of parametric analyses comparing the performance of the hemispherectomy groups and the (NC) group indicated significant differences between group means (overall accuracy: F = 4.39, $P < 0.033$; trials without a correct alternative excluded: F = 12.40,

$P < 0.001$). Consistent with previous analyses, post hoc comparisons revealed that the (L-X) group was significantly impaired relative to both the (R-X) and (NC) groups.

Examination of the scores for individual patients within the (L-X) group did not reveal any trends highlighting the effects of the variable of age at injury. Whereas the same absence of trends was also apparent in the accuracy scores for the (R-X) groups for trials without a correct alternative, there were indications that overall accuracy was most deficient for the (ECD, R-X) patient. In each condition, her performance was nearly 3 and 4 SDs below the means of the (NC) and (LT) groups, respectively.

Comparison of words vs nonwords. In order to examine simultaneously the effects of groups and task condition (words vs nonwords) on performance, separate two-way analyses of covariance (repeated measures) were carried out. The first analysis concerned overall accuracy scores while the second used accuracy on trials wherein a correct alternative was present. Results of both analyses indicated significant main effects of group (overall accuracy: $F = 8.38$, $P < 0.004$; accuracy excluding trials without a correct alternative: $F = 11.98$, $P < 0.001$) and of task condition (overall accuracy: $F = 51.54$, $P < 0.001$; accuracy excluding trials without a correct alternative: $F = 22.62$, $P < 0.001$). In neither analysis did the interactions of group by task reach significance. Post hoc analyses carried out on the pooled means of the groups indicated that for both measures performance on words was significantly better than on nonwords. Comparisons of group means (pooled over task conditions) showed that for both measures, the (L-X) group was significantly impaired relative to both (R-X) and (NC) groups.

DISCUSSION

Effects of side of hemispherectomy

Baseline language measures. The present data suggest that in cases of early injury, the isolated right hemisphere is as capable as the isolated left hemisphere in subserving aspects of everyday verbal communication (e.g., verbal IQ, MQ) and specific domains of language processing (e.g., auditory language comprehension), even though these capacities have been shown to be selectively sensitive to left hemisphere injury in adults (De Renzi and Vignolo, 1962; Basso *et al.*, 1981). At the same time, the results also clearly indicate that, despite comparable intellectual and memory capacities in the two hemispherectomized groups, there are deficits in selective aspects of language production and comprehension in the (L-X) group relative to the (R-X) and other neurologically impaired control groups. The present findings support the conclusion that the efficacy of the isolated right hemisphere in gross language functions is not substantially worse after surgery than before (McFie, 1961; Beardsworth and Adams, 1988). Consequently, the deficiencies of this hemisphere cannot be attributed to the physical removal of the left hemisphere but must reflect its innate inadequacy.

As to the nature of the selective language deficits in the (L-X) group, the impairment in naming to confrontation reflects the limitations of the right hemisphere in providing efficient lexical access; this finding is consistent with the well established notion that expressive disorders are a consequence of dominant hemispherectomy. Similarly, it is interesting to note that with regard to the errors of the (L-X) patient on the TROG test, none of the cases passed all 4 of the items (nos 45–48) constituting Block L, which is designed to assess comprehension of the affirmative passive construction. By way of comparison, none of the (R-X) patients experienced difficulties in comprehending such structures, even though the (MCD, R-X) cases' first error appeared on a less difficult item (no. 39). Passive sentences thus reveal the grammatical limitations of the isolated right hemisphere. This impairment is consistent with many previous findings (Dennis and Whitaker, 1976; Ludlow, 1980). However, it is inconsistent with the later finding of Dennis and Whitaker (1976) of a selective impairment in the comprehension of passive negative sentences only, though it should be pointed out that in a more recent study, Dennis (1980) subdivides left hemispherectomy patients into a group that does not and another that does show decoding deficiency of passive affirmative sentences as well. As already indicated, these selective impairments in the (L-X) group are not simply a consequence of lowered intelligence and/or memory capacity, in as much as the (R-X) group, with equivalent IQ and MQ scores, was nevertheless unimpaired on the same selective language abilities relative to the other control groups. This strong conclusion must be modified somewhat when performance of the (R-X) patients is considered case by case as a function of age at injury. A later section of the discussion will address this issue.

Production and comprehension of morphological markers. In addition to their impairments on baseline measures, the (L-X) group exhibited difficulty with morphological markers. While the comparisons between the hemispherectomy and control groups revealed the pervasiveness of the (L-X) group's deficit in production of morphological markers, the crucial comparisons are again those between left and right hemispherectomized groups. From these comparisons, it is clear that the isolated right hemisphere can produce such markers efficiently as long as it has access to cues provided by familiar semantic and syntactic contexts. Thus, the performance of the (L-X) and (R-X) groups did not differ significantly when inflections of real words are required. However, deprived of access to such cues, as for sentences containing a nonword, the isolated right hemisphere is deficient in using the degraded semantic and/or syntactic cues to produce correct inflections. All patients were tested on the ability to repeat nonwords and had no difficulty in doing so. Fundamentally, this deficit appears to reflect a limitation in the right cerebral hemisphere's 'internal representation' of English morphology.

The same limitation was not found in the (R-X) patients, although at least in one condition (production of morphological markers for real words) they had a significant deficit relative to the normal control group. In this task, the performance of the (R-X) group fell between that of the (L-X) and normal control groups. The finding of a mild degree of language production deficit is consistent with other reports of the effects of right hemispherectomy consequent upon early onset injuries (McFie, 1961; Basser, 1962).

The pattern of results on morphological judgements was entirely consistent with those obtained on the production version of the task. In this task also, the two hemispherectomized groups cannot be differentiated on the basis of recognition of real word markers, suggesting that at least the surface aspects of morphological decoding are equally preserved in the (L-X) group. Furthermore, the (L-X) patients could not be differentiated from the (R-X) patients under the condition in which both markers were incorrectly inflected, regardless of whether the markers were words or nonwords. The (L-X) group was relatively deficient, however, when trials without the correct alternative were excluded. This result is probably a reflection not of a decrease in performance of the (L-X) group, but rather of an increase in the performance of the (R-X) group, which was better able to take advantage of the reduced ambiguity. Another indication of this improvement in the (R-X) patients was the finding that their scores became less variable, the SD being reduced to less than half of what it was when the trials without a correct alternative were included.

The effects of age at injury

Left hemispherectomy cases. The degree of selective language impairment after left hemispherectomy remained roughly constant, irrespective of age at injury for some measures but not all. For example, among the baseline language measures, the number of blocks passed on the TROG test and accuracy on the Token Test was about the same for all (L-X) cases. On other baseline measures, however, impairments appeared to vary as a function of the age at which the initial insult was sustained. This was true for accuracy of naming to confrontation, reaction time for naming, and the total number of errors committed on the TROG test. On all of these measures, the pattern of scores suggested that the earlier the left hemisphere injury the less pronounced the deficit.

A similar set of findings, with age at injury irrelevant on some measures but relevant on others, was obtained on the experimental tasks. For example, on overall accuracy of morphological production of words and nonwords and on judgement of morphological markers of nonwords (and words when trials without a correct alternative were excluded), impairment was not clearly related to age at insult. On the other hand, accuracy of producing inflectional markers for both words and nonwords and judgement of morphological markers for words tended to vary with age of insult, the impairments again being less pronounced the earlier the onset of injury. Among all the measures, the only instance in which the reverse trend was seen, i.e., a greater impairment the earlier the onset of injury, was for accuracy in the production of derivational markers. In short, in nearly all tasks in which degree of impairment in the (L-X) group varied as a function of age at insult, greater though never complete sparing was seen when the injury occurred early. The same conclusion applies beyond selective language impairments per se to preservation of verbal as compared with full scale IQ following early left hemisphere insult.

Right hemispherectomy cases. For these cases, the present data indicate that if the initial insult is acquired from the middle childhood period (between 5 to 6 yrs of age onwards), language functions remain largely unaffected. The same, however, cannot be said of the (ECD, R-X) case whose initial insult occurred at 2 yrs of age and whose baseline language performance was below that of the other (R-X) patients. In fact, with a preoperative verbal IQ of 55 and performance IQ of 73, and a baseline language screen suggestive of verbal comprehension deficits, the (ECD, R-X) case resembled the (L-X) cases more than the other two (R-X) cases. Similarly, whereas the performance of the early (R-X) case on the morphological tasks was above that of the (L-X) cases, it was nevertheless below the performance of the other (R-X) case.

The foregoing analysis must remain tentative, however, in as much as it rests on the results of a single case. Yet it would be unwise to dismiss the possibility that early extensive damage to the right hemisphere limits the capacity for language development at least until there is an opportunity to study further cases.

Effects of early diffuse seizure activity

In this investigation, age at injury was either defined as age at onset of seizure activity or was closely related in time to seizure onset. The contribution of seizure activity to the observed impairments cannot therefore be ignored. Some insight into the influence of this variable is provided by a comparison of the performance of the (L-X) group and the (LC) group, since the latter sustained early extensive left hemisphere damage in the absence of concomitant seizure activity. It is of interest that on all language measures used in this investigation, the performance of the (LC) group was indistinguishable from that of the (NC) group and consequently differed equally from the (L-X) group. The absence of a deficit in the (LC) group is in contrast to an earlier finding involving another group of infantile hemiplegic patients with left hemisphere insults and concomitant diffuse seizure activity, whose performance on the same baseline language measures was significantly impaired relative to the normal control group (Vargha-Khadem *et al.*, 1985).

The conclusion to be derived from these findings is not contradicted by the normal language performance of the (LT) group. First, their seizure activity was focal and confined to the left temporal lobe. Secondly, the mean onset of their focal seizures was at age 5.9 yrs, only 2 patients having suffered seizures before the age of 3 yrs. Finally, neither their focal seizures nor their surgical excisions would be expected seriously to interfere with the development of language functions unless they also place heavy demands on memory processes.

Although no final conclusion regarding the role of early diffuse seizure activity on language development can be drawn from the present data, the normal performance of the (LC) group clearly suggests that such seizure activity exerts a major deleterious influence. Thus it may be the case that the combined effects of side of hemisphere insult, age at injury and presence or absence of diffuse seizures together determine the pattern and course of development of language functions during childhood.

ACKNOWLEDGEMENTS

We thank Karalyn Patterson for her encouragement and constructive comments and Max Coltheart for discussing ideas on the morphological tasks. We thank Maureen Lovett for providing us with details of the Morphological Production Paradigm and its modifications. We also thank the families and the patients who tolerated the long journeys necessary for their visits and the patients for their cooperation and patience. The preoperative psychometric testing was undertaken by the clinical psychology service of the Maudsley Hospital. This research was supported by the Medical Research Council.

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(*Received September 6, 1989. Revised March 21, 1990. Accepted April 3, 1990*)

APPENDICES

Appendix 1

In the modified surgical procedure introduced by Adams (1983) precautions are taken to avoid long-term complications often associated with the standard hemispherectomy operation. To prevent subdural collection, following the removal of the diseased hemisphere, the convexity dura is sutured down to the falx, tentorium and floor of the anterior and middle cerebral fossae. In addition, a plug of muscle is inserted into the ipsilateral foramen of Monro, thus isolating any residual subdural collection from the ventricular system. As a result of these procedures, the hemispherectomy space is principally filled by an extradural as opposed to a subdural collection.

Total hemispherectomy refers to the removal of the cortex of one cerebral hemisphere, along with varying portions of the ipsilateral basal ganglia, subtotal hemispherectomy normally involves the removal of approximately 2/3 of the damaged hemisphere leaving intact some functional cortex, most typically the posterior third of the hemisphere

Appendix 2

We have also investigated 7 other patients who have undergone hemispherectomy consequent upon acquired insults of childhood (4 left, 3 right). Consistent with the pattern of performance of the present cases, none of the left hemispherectomy patients passed the passive sentences on the TROG test whereas all right hemispherectomy cases did so without difficulty.

Appendix 3

PREOPERATIVE AND POSTOPERATIVE WECHSLER IQ SCALED SUBTEST SCORES OF THE HEMISPHERECTOMY PATIENTS

	Verbal											
	Information		*Comprehension*		*Arithmetic*		*Similarities*		*Digit Span*		*Vocabulary*	
Case	*Pre*	*Post*	*Pre*	*Post*	*Pre*	*Post*	*Pre*	*Post*	*Pre*	*Post*	*Pre*	*Post*
1	–	6	–	3	–	4	–	6	–	4	4	3
3	–	3	–	4	–	4	–	5	–	2	–	5
5	–	6	1	3	–	1	–	1	–	3	1	–
2	3	3	–	5	3	3	3	2	5	2	–	0
4	–	5	–	5	–	5	12	5	–	4	5	–
6	–	5	5	6	–	5	7	11	–	2	6	5

	Performance									
	Digit Symbol		*Picture Completion*		*Block Design*		*Picture Arrangement*		*Object Assembly*	
Case	*Pre*	*Post*	*Pre*	*Post*	*Pre*	*Post*	*Pre*	*Post*	*Pre*	*Post*
1	–	4	–	3	1	7	–	4	–	3
3	–	4	–	3	–	7	–	5	–	7
5	–	1	–	4	4	9	–	7	1	7
2	3	2	6	5	7	4	–	–	7	5
4	–	4	–	3	5	1	–	–	4	3
6	–	6	–	5	7	10	–	4	2	5

Pre = preoperative, Post = postoperative

Appendix 4

WECHSLER IQ SCALED SUBTEST SCORES FOR THE TEMPORAL LOBECTOMY (LT) AND THE CONGENITAL HEMIPLEGIC (LC) CONTROL GROUPS. MEANS AND SDs

	Information		*Comprehension*		*Arithmetic*		*Similarities*		*Digit Span*		*Vocabulary*	
Groups	$\bar{X}$	*SD*	$\bar{X}$	*SD*	$\bar{X}$	*SD*	$\bar{X}$	*SD*	$\bar{X}$	*SD*	$\bar{X}$	*SD*
LT (preoperative)	7.0	1.4	6.8	1.2	6.5	1.1	8.4	2.4	6.0	2.4	6.3	2.2
LC	10.1	2.2	10.7	3.9	10.8	3.2	10.6	2.7	9.4	3.1	8.2	3.9

	Digit Symbol		*Picture completion*		*Block Design*		*Picture Arrangement*		*Object Assembly*	
Groups	$\bar{X}$	*SD*	$\bar{X}$	*SD*	$\bar{X}$	*SD*	$\bar{X}$	*SD*	$\bar{X}$	*SD*
LT (preoperative)	7.5	2.1	10.0	7.2	9.6	2.3	–	–	8.3	2.4
LC	9.1	2.6	8.4	2.1	10.2	3.2	9.3	3.3	8.9	2.8

Brain (1991), **114**, 497–512

TARGET DETECTION AND MOVEMENT DISCRIMINATION IN THE BLIND FIELD OF HEMISPHERECTOMIZED PATIENTS

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SUMMARY

Four hemispherectomized patients were tested with a variety of tasks designed to investigate the extent of residual vision in the blind field. The first set of studies was aimed at evaluating the ability of these subjects to detect and localize at various eccentricities three types of targets which differed in their spatio-temporal properties (stationary, flashing and moving). The subjects could detect effectively the presence of any of these stimuli in their blind field and they experienced little difficulty in discerning blank from target trials When manual pointing was used to measure localization quality, the hemianopes made more errors in their blind field but their accuracy, when detection was correct, was comparable with that observed in their intact visual field and in that of the control subjects. In the second set of experiments, the capacity of 3 of the subjects to detect in their blind field a moving grating, as well as to discriminate between relative grating velocities and directions in their blind field and in both fields simultaneously, was assessed. Two subjects could detect the movement in their blind field, although this was in part affected by stimulus velocity. When discrimination of relative velocities in the blind field and in both fields simultaneously was evaluated, individual differences were observed. However, all subjects showed some capacity to carry out the task. Finally, none of the experimental subjects was able to discriminate the relative directions of the moving gratings. The results are discussed in terms of collicular involvement to account for the residual vision observed in the blind field of hemispherectomized subjects.

INTRODUCTION

Since Holmes' report (1918), it has become traditional to expect that a lesion of the occipital cortex leads to a permanent loss of vision in the corresponding part of the visual field. More recently, however, evidence has been accumulating that hemianopic subjects demonstrate residual visual capacities in the blind part of their visual field. The types of residual vision reported are wide ranging, and include saccadic and manual target detection and localization (Pöppel *et al.*, 1973; Weiskrantz *et al.*, 1974; Perenin and Jeannerod, 1978; Stoerig and Pöppel, 1986), form discrimination (A. Ptito *et al.*, 1987; Weiskrantz, 1986, *but see also* Weiskrantz, 1987, who attributes his subject's superior performance more to orientation than to actual form discrimination), chromatic and achromatic target detection (Stoerig, 1987), spectral sensitivity (Stoerig and Cowey, 1989), pupil reactivity to light changes (Brindley *et al.*, 1969) and induction of optokinetic nystagmus (ter Braak *et al.*, 1971). While it has been argued that spared cortical areas

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and/or methodological artefacts may have been responsible for such residual capacities (e.g., Campion *et al.*, 1983), several findings indicate otherwise (*see* Weiskrantz, 1986, for a review).

Experiments on the interhemispheric modulation of light-difference thresholds in normal subjects and in hemianopic patients (Singer *et al.*, 1977), as well as on a patient with a congenital malformation of the right superior colliculus (Zihl and von Cramon, 1979*a*), support the view that the latter structure contributes to residual vision in the blind field. This notion has been reinforced by results obtained with hemicorticectomized subjects. Perenin and Jeannerod (1978) specifically attributed to the collicular pathway the ability of these subjects to localize visual stimuli in their hemianopic field, since the lateral geniculate body and the pulvinar are known to degenerate following hemispherectomy (e.g., Ueki, 1966).

Several lines of converging evidence tend to support this assumption. Mohler and Wurtz (1977) have demonstrated that the residual detection abilities observed in striatectomized monkeys disappear after collicular lesions. The superior colliculi of the monkey have been shown to receive visual input from the retina, as well as striate cortex (Schiller, 1972; Perry and Cowey, 1984) and they contain a complete representation of the visual field (Schiller, 1972). Furthermore, cells in the superficial layers of the monkey superior colliculus respond to flashing, moving or 'jerking' displacements of visual stimuli (Moors and Vendrik, 1979), while their stimulation in the intermediate layers triggers a saccadic eye movement towards the specific region in the visual field corresponding to its retinotopic representation (Robinson and Wurtz, 1976).

Several of these studies suggest that the superior colliculi contribute to qualitatively different aspects of vision than the cortical visual areas. The electrophysiological studies cited above (Robinson and Wurtz, 1976; Moors and Vendrik, 1979) are some examples. Patients with midbrain pathology show reduced latency of orienting responses (Heywood and Ratcliff, 1975; Posner *et al.*, 1982) and collicular lesions in animals impair orientating responses towards visual stimuli, reduce responsiveness to novel stimuli (Schneider, 1967; Goodale and Murison, 1975; Milner *et al.*, 1978) and produce deficits in movement discrimination (Anderson and Symmes, 1969; M. Ptito *et al.*, 1976; Collin and Cowey, 1980).

Considering these facts, the goal of the present study is to extend previous results on the existence of residual vision in the blind field while carefully controlling for possible methodological artefacts. The use of subjects that have undergone the complete removal of a whole cerebral hemisphere is important because it allows rejection of the argument that residual vision is subserved by spared striate cortex. As a corollary to this, these subjects permit the testing of the visual processing capacities of remaining midbrain structures such as the superior colliculi, since these are the structures which are probably responsible for residual visual abilities. Stimuli were therefore chosen which were appropriate for collicular functions, such as stimulus detection and localization as well as movement detection and relative velocity discrimination. The first set of studies was aimed at evaluating the ability of the subjects to detect and subsequently to localize 3 targets with differing spatiotemporal properties and used briefly presented stimuli which were either stationary, flashing or moving. The second set of experiments assessed the ability of the subjects to perceive movement in tasks of increasing complexity. In this context, we investigated the subjects' capacities to detect, in their blind field and in both fields simultaneously, the movement of a grating, to discriminate relative velocity differences and to compare relative directions of movement.

METHODS

Subjects

The experimental group was composed of 4 subjects (Table 1) who had previously undergone complete cerebral hemispherectomies (3 left, 1 right) for the relief of uncontrollable epileptic seizures. All patients suffered from severe head injury that occurred shortly after birth, except for Case 4 who developed seizures at age 2.5 yrs following an episode of encephalitis. Surgery involved complete cortical removal on one side, sparing the thalamus and caudate nucleus. Age at operation varied between 8 and 14 yrs. All subjects were submitted to a complete neuropsychological assessment, Goldman perimetry and CT scanning and the results are reported in detail in a previous publication (A. Ptito *et al.*, 1987). Briefly, all subjects were in the mildly defective range of intelligence. Within the experimental group, Cases 1 and 3 were better on perceptual or visuomotor tasks whereas 2 and 4 had a superior performance on verbal tasks. Goldman perimetry demonstrated the presence in all cases of a complete hemianopia without macular sparing in the field contralateral to the removal. Finally, CT scans confirmed total hemisphere removal and showed marked ventricular dilatation in the remaining cerebral hemisphere as well as its shift towards the cranial cavity. In spite of the fact that each subject could serve as his/her own control by comparing performances in the blind and in the intact field, 2 control subjects matched for age, sex and IQ also participated in the studies. The latter were included to ensure that any difficulty in performing the tasks was due to perceptual and not intellectual limitations.

Apparatus and stimuli

All experiments were carried out monocularly with the subjects using their dominant eye as established through visual acuity examination and standard ocular dominance tests. The subject, with the nondominant eye covered, was seated 1.14 m from the central fixation point in an adjustable hydraulic chair fitted with a head retainer and a chin holder that permitted reliable immobilization of the head and maintenance of constant eye level. Continuous monitoring of eye movements was achieved by means of Beckman EOG electrodes placed on the temporal periphery of the external orbit of each eye. Any horizontal movement of the eyes produced an electrical signal which was then amplified and transmitted to an Apple II microprocessor. The following calibration procedure was used to ensure eye fixation during stimulus presentation. (1) Three light-emitting diodes (LEDs) were placed at −30°, 0°, +30° at eye level above a semicircular table (radius: 114 cm); (2) the subject, head immobilized, was required to direct his/her gaze successively to each LED; (3) at each of these eccentricities an arbitrary value was assigned and recorded by the microprocessor; (4) the sum of these values, which represent the subject's visual field from −30° to +30°, allowed the experimenter to find the interpolated value corresponding to the maximal range of ocular movements permissible (5°), (5) any ocular movement exceeding this value interrupted stimulus presentation and produced the automatic rejection of the trial in which it occurred; the trial was subsequently repeated at the end of the session. In addition to the constant monitoring of eye movements, a supplementary procedure was used to facilitate fixation. The subject was instructed to fixate a centrally presented vertical row of 8 randomly flickering lights superimposed at intervals of 2.5 cm (total height: 18 cm) and to tap on the table as soon as one of the LEDs remained on. The tapping response was picked up by a microphone and relayed to the microprocessor, which then triggered, within 5 ms, the presentation of the stimulus. Subjects were dark-adapted for 10 min. In order to ensure that potentially distracting stimuli, as well as stimulus manipulations, were not visible to the subject, all experiments were carried out in a specially designed dark room. Between each trial and before giving his/her response, a curtain was pulled in front of the subject. To allow stimulus changes, moreover, an ultraviolet light was turned on. In none of the experiments were the subjects given feedback about the exactness of his/her performance.

All stimuli (light intensity 16 cd/m^2, contrast value 82.8%, background 1 5 cd/m^2) were presented on 2 video monitors (Sony Trinitron, 48 cm diagonal) mounted on wheeled platforms attached to the base of a semicircular track, which constituted the outer limit of the perimeter table (radius: 1.14 m). The midline of the chair used by the subject was placed in the centre of this semicircular table. The wheeled platforms carrying the monitors could be moved silently about the semicircular table at designated eccentricities. As a supplementary control for possible localization by sound, both monitors were always displaced even if the stimulus appeared on only one of them or on neither ('blank' trials). In addition, the left and right video monitors were never placed mirror-symmetrically on each trial in order to avoid target localization in the blind field by pointing to the corresponding position in the intact field. Furthermore, since the subject could see neither his hand nor the monitor he could not modify his response using extraneous cues.

Experiment 1. Visual localization of briefly presented targets

Three types of stimuli, presented briefly during 150 ms in the centre of the video monitors, were used in this experiment: a stationary square, a flashing square (3 flashes within 150 ms) and a moving square (2° vertical displacement; 0-down-0: 150 ms). Stimulus size (2° visual angle) remained constant and stimulus positions were established randomly at 8 eccentricities (5°, 20°, 35° and 50°), 4 to the left and 4 to the right of the fixation point. To eliminate as much as possible any persisting after-image and to reduce light scatter, neutral acetate filters were used to cover the screens. In addition, a black cardboard was placed on the unused screen in order to cut out any possible reflection. The total number of trials for Experiment 1 was 180, 60 for each type of stimulus (stationary, flashing, moving), 20 left, 20 right and 20 'blanks' in which no stimulus was presented. After each presentation, the subject had to maintain fixation and indicate whether or not a stimulus had been presented. In the affirmative, he was required to point with the index finger of the hand ipsilateral to the removed hemisphere the location where he/she thought the stimulus had appeared. The eccentricity to which the hand was pointing represented the subject's response. Lines representing the various eccentricities were clearly marked on the semicircular table at 5° intervals such that it was possible to assign a numerical value to a subject's response by aligning the hand with the closest marking on the table.

Experiment 2. Movement detection

A single monitor and a split-screen technique where 1 stimulus was presented above and 1 below the horizontal axis, were used for intrafield discriminations For bilateral presentations, 2 screens were used and the unused half screen was covered to eliminate possible reflections. The height of the monitors was adjusted so as to present the gratings at the level of the fixation point (bilateral presentations) or on symmetric distances from this meridian point when intrafield comparisons were carried out. The stimuli consisted of sinusoidal spatial frequencies of equal size (0.3 cycles/deg), always presented at 20° on either side of the fixation point. Presentation of the stimuli was provided by an Apple II microprocessor during 3 s. The method of monitoring eye movements described above was used and a maximum ocular movement of 5° was accepted.

Four conditions were used in this experiment: (1) *movement detection*: the subject had to indicate whether there was movement of the grating or not in one field or the other Four types of stimuli were used here: a rapidly moving grating (2.6 cycles/s, 40 trials), a slowly moving grating (0.3 cycles/s, 40 trials) a stationary grating (40 trials) and an absence of stimulus (40 'blank' trials), (2) *relative velocity discrimination*: the subject had to compare the speed of the 2 moving gratings and to indicate if they moved at the same or at different speeds. All 9 possible combinations between rapid movement (2.6 cycles/s), moderate movement (0.6 cycle/s) and slow movement (0.3 cycle/s) were presented. The presentations of the pairs of stimuli were made in blocks of 18 trials, first in the intact field, then in the blind field and finally in both fields simultaneously. There were therefore 162 trials in total; (3) discrimination of relative *direction of movement*: this was assessed under four conditions—the grating displacements were in the same (left, left; right, right) or opposite directions (left, right; right, left). Each of the four conditions comprised 36 trials (12 in the left, 12 in the right and 12 in both fields) for a total of 144 trials. The subject had to indicate whether the gratings moved in the same or in different directions starting with the intact field, then in the blind field and finally in both fields simultaneously.

RESULTS

Experiment 1

The first experiment was designed to investigate each subject's capacity to detect and localize targets (stationary, flashing or moving) by pointing manually. The stimuli were presented briefly either in their blind field or in their intact visual field. Blank trials were interjected randomly.

Target detection. Table 1 summarizes the performance demonstrated by each subject on the detection of targets. The control subjects, matched for IQ, obtained perfect or near-perfect scores in both visual fields and in all conditions. For the experimental subjects, an error was assigned when the subject denied the presentation of the stimulus or when the pointing response was made in the field opposite to that of stimulus presentation. Percentage success thus represents the proportion of pointing responses in the appropriate field of presentation. A performance of 37 correct responses out of 60 (62%) differed from chance at the 0.05 level according to a binomial distribution.

All experimental subjects demonstrated an excellent capacity (80 – 100%) to differentiate between 'blank' trials and real stimulus presentations. They also reliably detected (88 – 100%) the presence of the visual targets in their intact field. In addition, the subjects rarely denied that a stimulus was presented in their intact field and only in very few instances did they perceive the source of stimulation as being in the other field.

In their blind field, all patients, except Case 4 under only one condition (flashing), could detect significantly the presence of the targets. The proportion of trials in which they denied stimulus presentation was comparable with that observed in the intact field, with the exception of Case 4 who denied the presence of a stationary target in 36% of the trials. The most common error consisted of pointing responses in the opposite field of stimulation. This behaviour may be linked to the fact that these subjects, although aware of stimulus presentation (typical comment: 'I know there is something there!'), were guessing the origin of the stimulus. Case 3, however, showed none of the above behaviours; in fact, he obtained a perfect performance under all conditions.

Localization of targets. The accuracy of localization was assessed by calculating the mean deviation of the pointing responses from the target positions (5°, 20°, 35°, 50°). Obviously this computation was performed only on those trials in which the subject detected the stimulus on the correct side. The performance, in terms of precision of localization between the blind and intact fields of the experimental subjects, was submitted to an ANOVA with repeated measurements. This analysis was conducted on the mean deviation of the pointing responses at each eccentricity and for each type of stimulus (stationary, flashing and moving). No significant differences were found between fields of presentation ($F(1,3) = 0.267$, $P = 0.641$). No differences in accuracy were found in relation to type of stimulation or target eccentricity ($F(2,6) = 0.510$, $P = 0.6241$). The analysis showed, however, that the subjects tended to localize less precisely, though without attaining statistical significance, the stationary stimuli presented in their blind field ($F(2,6) = 3.353$, $P = 0.1$).

Since no significant differences were found between the types of target used, results obtained under the 3 modes of stimulation were combined. The performances of the control and experimental subjects thereby derived are illustrated in the fig. This shows a close correlation between target location and pointing in either field. Control subjects were very precise in both hemifields as they rarely undershot or overshot the target by more than 10°. Experimental subjects were also accurate in both their intact and blind visual fields. With the exception of Case 4, they generally showed a tendency to overshoot the targets presented in their blind field, but this pattern of response was also present, to a lesser degree, in their intact field.

TABLE 1 PERCENTAGE OF SUCCESS AND ERRORS IN DETECTING STATIONARY (S), FLASHING (F) AND MOVING (M) TARGETS IN EACH HEMIFIELD

					Intact field			*Blind field*		
Case/subject	*Eye used*	*Blind field*	*Blanks detected (%)*		*Denial (%)*	*Displacement in other field (%)*	*Correct (%)*	*Denial (%)*	*Displacement (%)*	*Correct (%)*
1 (F C)	R	R	80	S	0	6	94	0	29	71
			85	F	0	0	100	0	35	65
			80	M	0	0	100	0	10	90
2 (L.E.)	R	R	80	S	0	12	88	5	15	80
			100	F	6	0	94	0	9	91
			100	M	6	6	88	5	19	76
3 (D V)	R	L	100	S	0	0	100	0	0	100
			100	F	0	0	100	0	0	100
			100	M	0	0	100	0	0	100
4 (F G.)	R	R	100	S	6	0	94	36	0	64
			100	F	5	0	95	5	35	60*
			100	M	0	11	89	0	32	68
5 (R L., control)	L	–	100	S	0	0	100	0	6	94
			100	F	0	0	100	0	0	100
			100	M	0	0	100	0	0	100
6 (P M , control)	R	–	100	S	0	0	100	0	0	100
			100	F	0	0	100	0	0	100
			100	M	0	0	100	0	0	100

* Not significant (below chance).

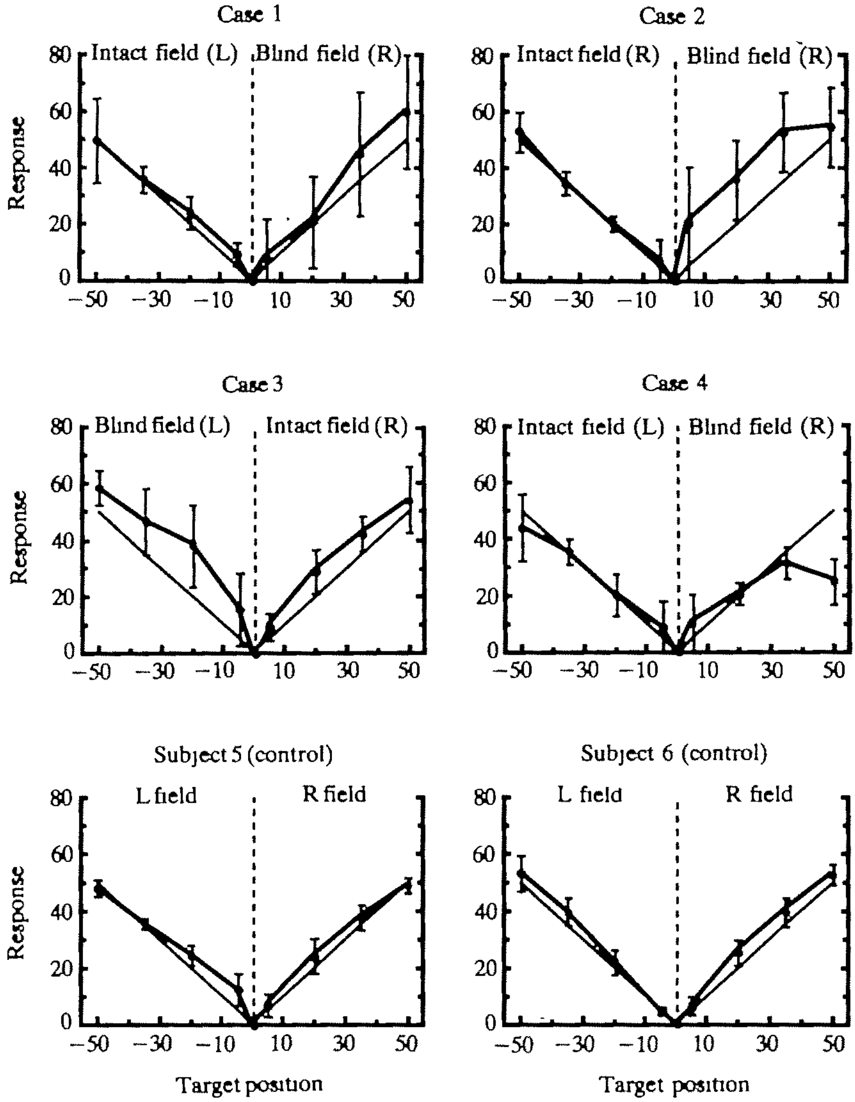

FIG Accuracy of localization of targets (results for stationary, moving and flashing stimuli are combined) in each hemifield for control and hemispherectomized subjects. Thin straight line = expected values, filled dots = actual values obtained by the subjects; vertical bars = 1 SD from mean value

Experiment 2a. Movement detection

Only 3 experimental subjects participated in this experiment; Case 4 refused because he found the task too demanding. In this part of the experiment, the subject's task was to detect the presence or absence of a grating and, in the affirmative, he had to report if it was moving or not. Four conditions were used: (1) blank trials, and trials in which the grating was (2) stationary, (3) slowly moving, and (4) rapidly moving. The results are presented in Table 2.

TABLE 2 PERCENTAGE CORRECT RESPONSES ON MOVEMENT DETECTION OF GRATINGS

	Stationary		*Slow*		*Rapid*		
Case/Subject	*IF*	*BF*	*IF*	*BF*	*IF*	*BF*	*Blank*
1	90	20*	30*	10*	90	10*	100
2	100	65	100	35*	100	65	100
3	95	93	85	5*	100	95	100
5 (control)	100	100	98	100	100	100	100
6 (control)	98	100	93	100	100	100	100

* At or below chance level. IF = intact field, BF = blind field.

As indicated in Table 2, all subjects detected without error the 'blank' trials. The control subjects obtained over 93% correct responses in all experimental conditions. A performance of 26 correct responses out of 40 trials (65%) differed from chance at the 0.05 level according to a binomial distribution. The experimental subjects also demonstrated an excellent performance in their intact visual field although Case 1 showed difficulty in detecting a slowly moving grating. In the blind field, individual differences were noted. Even though capable of identifying 'blank' trials, Case 1 was unable to indicate whether the grating was stationary or moving. In contrast, Case 2 was able to differentiate between a stationary and a rapidly moving grating in her blind field. The results for Case 3 compared favourably with those of Case 2 as he was also able to detect when a grating was stationary and when it was moving rapidly. It is noteworthy that the detection of a slow movement in the blind field could not be carried out by any of the experimental subjects. In summary, all experimental subjects were capable of detecting the presence of a grating and 2 out of 3 were able to distinguish between the rapidly moving grating and a stationary one. All subjects experienced problems with the detection of a slow movement. These difficulties may be attributed to the velocity of the slowly moving stimulus which may have been too difficult to discern from an immobile one. This possibility is supported by the poor performance of Case 1 with this type of stimulus presented in his intact field.

Experiment 2b. Relative velocity discrimination

In this experiment, 3 different velocities were used: rapid, medium and slow. The possible combination of velocities yielded 6 discriminative pairs (rapid-rapid; medium-medium; slow-slow; rapid-medium; medium-slow; rapid-slow).

For statistical purposes, the results were grouped according to the relative difference in velocity between stimulus pairs. Thus, a 'large' difference comprised the pairs rapid-slow while a 'median' difference was made up to the pairs rapid-medium and medium-slow. The 'no-difference' condition was composed of the pairs rapid-rapid, medium-medium and slow-slow. The subject's task was to indicate if the gratings moved at the same velocities or if their rate of displacement was different. The results are presented in Table 3. A performance of 13 correct responses out of 18 trials (72%) differed from chance at the 0.05 level according to a binomial distribution.

TABLE 3 PERCENTAGE SUCCESS IN DISCRIMINATING VELOCITY DIFFERENCES

	Velocity difference								
	Intact field			*Blind field*			*Both fields*		
Case/Subject	*Same*	*Median*	*Large*	*Same*	*Median*	*Large*	*Same*	*Median*	*Large*
1	83	79	92	20*	42*	67*	56*	41*	25*
2	89	92	92	30*	83	75	29*	67*	75
3	100	58*	100	94	29*	50*	94	42*	75
	Left field			*Right field*			*Both fields*		
5 (control)	100	100	100	100	96	100	100	96	100
6 (control)	100	96	100	100	96	100	100	58*	100

* At or below chance level.

Results presented in Table 3 indicate that the control subjects obtained over 96% correct responses in all conditions except one. In the 'median' difference condition, subject 6 could not discriminate a difference in grating velocity when the stimuli were presented simultaneously in both hemifields. A similar difficulty was observed in the intact field of Case 3. This result is difficult to explain other than by an incidental decrease in attention. Generally, however, the performance of the experimental subjects in their intact field was good and comparable to those of the control subjects.

In the blind field, individual differences were predominant. Case 2 was able to discriminate 'large' and 'median' differences in velocities almost as efficiently as normal subjects but she remained at chance when the gratings moved at the same speed. In contrast, Case 3 was able to detect an absence of difference between velocities but he was unable to discriminate between 'large' and 'median' differences. Finally, the performance for Case 1 remained at chance in all conditions involving his blind field. When the gratings were presented simultaneously in both hemifields, similar results were obtained. Performance in Case 1 remained at chance while Case 2 was able to detect 'large' differences. Case 3 could discriminate an absence of difference between velocities and he was also able to detect extreme differences between the gratings.

Experiment 2c. Discrimination of movement direction

In this experiment, the subjects had to report whether the direction of displacement of the stimuli presented in the intact field, in the blind field or in both fields simultaneously were the same or different. The results are presented in Table 4. A performance of 24 correct responses out of 36 (67%) differed from chance at the 0.05 level according to a binomial distribution of success. This table shows that the control subjects demonstrated perfect performance in all conditions. Experimental subjects obtained over 90% success for discriminations in their intact visual field. But when presentations were made in the blind field or in both fields simultaneously, all the experimental subjects' performances dropped to chance level.

TABLE 4 PERCENTAGE OF SUCCESS IN DISCRIMINATING THE DIRECTION OF MOVEMENT

Case/subject	*Intact field*	*Blind field*	*Both fields*
1	90	50*	54*
2	100	52*	50*
3	100	58*	46*
	Right visual field	*Left visual field*	*Both fields*
5 (control)	100	100	100
6 (control)	100	100	100

* At or below chance level.

DISCUSSION

The results reported in this study confirm the presence of residual vision in the blind field of hemispherectomized subjects. As previously noted by others (e.g., Perenin and Jeannerod, 1978; Weiskrantz, 1989) and ourselves (A. Ptito *et al.*, 1987), the extent and quality of this vision vary among subjects and type of task investigated. First, the detection in the blind field of a moving, flashing or stationary stimulus could be carried out effectively by all subjects. In addition, all hemispherectomized subjects experienced little difficulty in distinguishing blank trials indicating that the task was well understood and that they were not responding randomly to the presence or absence of the stimuli. When manual pointing was used as a measure of localization quality, the hemianopic subjects showed more errors in their blind field but their accuracy when detection was correct compared well with that observed in their intact field and with that of the control subjects. These results support those of several authors who reported that patients with geniculostriate lesions were able to detect and localize a target presented in their blind field either by orienting their eyes to the stimulus eccentricity (Põppel *et al.*, 1973; Zihl and von Cramon, 1979*b*; Barbur *et al.*, 1988) or by pointing to it (Weiskrantz *et al.*, 1974; Perenin and Jeannerod, 1978). Our results differ, however, from the previous studies on two grounds: the type of stimulus used did not differentially influence the performance and stimulus detection was common.

The first result pertaining to the undifferentiated nature of performance according to stimulus type may at first glance seem to be at odds with the conclusions of several authors (e.g., Weiskrantz *et al.*, 1974; Perenin and Jeannerod, 1978) who reported a greater precision in localizing flashing or moving targets. An explanation which could reconcile the seemingly contradictory findings is that we presented all our targets very briefly for 150 ms and we varied within this time frame the stimulus property (stationary, flashing or moving). It is therefore possible that under such limited exposure the subjects perceived all targets as being flashed.

The second observation concerned the frequency with which the subjects detected the stimuli on the correct side, even when this was the hemianopic field. The subjects, except for Case 4 in only one instance (stationary target), rarely denied that a stimulus had been presented. Moreover, they consistently identified blank trials (absence of the visual stimulus). They were therefore aware of the presence of a stimulus without, however, specifying its nature. This differs markedly from the observations reported by Weiskrantz *et al.* (1974) who used a forced-choice technique to circumvent their subjects' denial of the presence of a stimulus. In this context, we may have measured residual vision rather than 'blindsight' as described by the latter authors.

With regard to the extent of the discriminative capacity with moving gratings, our subjects were required to make a judgement within a forced-choice paradigm. With a prolonged 3 s stimulus presentation, all subjects but 1 (Case 1) could detect a moving grating in their blind field and we noted as have others (e.g., Blythe *et al.*, 1987) that different speeds of displacement may either facilitate or hinder the discrimination of relative velocities. All subjects, however, experienced difficulty in detecting a slow movement of 0.3 cycles/s, a displacement which may have been too difficult to distinguish from a stationary one. When discriminations of differences between movement velocities had to be carried out, all subjects showed some capacity to do them; again, individual differences in performances existed. Case 1 could not discriminate differences in velocities presented in his blind field; Case 2 was able to differentiate between median and extreme differences in speeds presented in her blind field but could only discriminate extreme differences in velocities presented in both fields simultaneously; Case 3 could differentiate extreme differences in speeds of gratings exposed in both fields concurrently and recognize equal speeds presented in his blind field or in both fields simultaneously.

The performance of the hemispherectomized patients in these discrimination tasks varied from that observed in the simpler target detection task. In spite of longer exposure times, the hemispherectomized patients were less accurate and they displayed a lower level of confidence when they gave their responses, most often reporting that they could only guess the differences in stimulus parameters. They were, however, consistently aware of the presence of the stimuli in their blind field and in that respect their behaviour contrasted to that observed in a previous study (A. Ptito *et al.*, 1987) aimed at studying shape and object discrimination. In the latter, not only was their level of accuracy in identifying the stimuli much lower than in the relative velocity discrimination task but in addition none of the patients ever reported having perceived the stimulus in their blind field. 'Blindsight', in its strictest sense, was therefore only observed in the object or shape discrimination task. With regard to the stimuli used in the present experiment, it may be more appropriate to discuss the hemispherectomized patients' abilities within the context of residual vision.

Of the possible explanations or mechanisms responsible for the residual vision reported here, several can be excluded. (1) *Light scatter*. We minimized the importance of this variable by covering the monitor screens with acetate filters. In addition, individual differences were clearly observed in all 3 experiments and no particular pattern of response which could be explained by light scatter was found. For example, in the movement detection experiment, all subjects were precise at the 5° eccentricity in localizing briefly presented stationary targets. According to a light scatter hypothesis, they should have been less precise with targets presented at more eccentric positions since in these cases light spread would be less important. This was not observed. In addition, in the movement detection experiment with prolonged stimulus presentation, Case 1 was unable to detect movement while the other subjects could detect an absence of movement or a rapid displacement but not a slow one. These differences cannot be accounted for by light scatter. Furthermore, in the trials where the stimuli were presented simultaneously in both fields, light scatter could not have been a factor responsible for the residual vision as the same amount of light emanated from both monitors placed in corresponding eccentricities on either side of the fixation point. Yet 2 subjects were able to carry out some interfield comparisons of displacement velocity; finally, none of the subjects could detect movement direction in their blind field in spite of the facts that the stimuli used were exactly the same as in the other experiments and that presentations were made at the same eccentricities. It is therefore concluded that the light scatter hypothesis cannot be retained to explain any of the results obtained here. (2) *Inadequate eye fixation*. Eye movements were monitored automatically and the computer program used ensured, through an interruption of the stimulus presentation in the presence of eye movements exceeding 5°, that the stimuli were lateralized. (3) *Spared striate cortex*. Hemispherectomized subjects were used in which there has been documented cortical ablation of the whole hemisphere.

The anatomical consequences of hemispherectomy have been reported, among others, by Ueki (1966) who examined microscopically the autopsied central nervous system of 2 hemicorticectomized subjects. Marchi's method was used for the demonstration of degenerated nerve fibres, and Nissl and Klüver-Barrera stains for studying retrograde degeneration. It was found that in addition to the absence of the whole cerebral hemisphere, there was retrograde degeneration of the entire thalamus, including the lateral geniculate body and the pulvinar. Because of the similarity in surgical interventions between these patients and our subjects, it can be postulated that the most probable candidate responsible for the residual vision in the blind field of our hemispherectomized subjects is the collicular system.

Support for the possibility that these midbrain structures contribute to residual vision in the absence of striate cortex was furnished in an experiment on the monkey by Mohler and Wurtz (1977). In a three-stage experiment, they first created a scotoma by making a small striate cortex lesion; they then gave extensive training and practice to the animal in detecting a briefly presented small flash of light. This resulted in a shrinking of the scotoma. Finally, by making a small lesion in that part of the superior colliculus to which the recovered portion of the visual field was known to project, they reinstated the scotoma permanently. According to the authors, the midbrain pathway was definitely involved in the recovery. Several other lines of evidence support this hypothesis. Anatomically, the superior colliculi receive projections from two of the three types of retinal ganglion cells (Y-like and W-like but not X-like; de Monasterio, 1978) and receptive field properties of collicular cells suggest a substantial convergence of retinal inputs onto the colliculus (Wurtz and Albano, 1980).

Collicular involvement in residual vision of hemispherectomized patients is further substantiated by comparing the responses obtained by our subjects in their blind field with the response characteristics of collicular cells. The contralateral visual field is mapped in an orderly way across the superficial layers of the superior colliculus (Cynader and Berman, 1972), a characteristic which could account for the spatial localization capacities reported by others (e.g., Perenin and Jeannerod, 1978) and observed in the blind field of our subjects. Collicular cells respond well to flashed or moving targets (Wurtz and Albano, 1980) and their sensitivity augments with an increase in target velocity (Barbur *et al.*, 1980). These properties are in keeping with the observations of Anderson and Symmes (1969) on colliculectomized monkeys who experience severe deficits in discriminating rate of movement, the implication being that the capacity to respond differentially to the displacement velocities of an object represents a specific aspect of visual function that is dependent on the integrity of the superior colliculus. The general pattern of our subjects' responses lends support to this explanation as all were able to some extent (albeit not all to the same degree) to carry out relative velocity discrimination in their blind field and/or in both intact and blind fields simultaneously. Moreover, they were better at discriminating rapid than slow movement.

We have thus far argued throughout that the residual vision observed in the blind field of the hemispherectomized patients is subserved by the superior colliculi. The stimuli were in fact chosen so as to excite maximally these nuclei and the results seem to support this hypothesis. A number of other structures, however, such as the pretectal nuclei, also receive visual inputs. Furthermore, the superior colliculi project to posterior thalamic nuclei, such as the pulvinar (Walker, 1938; Benevento and Standage, 1983; Jones, 1985; Marrocco *et al.*, 1981), whose cells have receptive fields which resemble collicular receptive fields in many ways (response to rapid movement, large receptive field sizes, etc.) (Bender, 1981, 1982; Peterson *et al.*, 1985). It is thus possible that these structures also contributed to the residual functions in conjunction with, or instead of, the superior colliculi. On the other hand, even if the colliculi are the principal structures involved in residual vision, their contribution has its limitations. In fact, it is known that receptive field properties of collicular cells are modified following the inactivation of the magnocellular portion of the lateral geniculate nucleus or its striate projection in the monkey (Schiller *et al.*, 1979). This results from the fact that the latter structure projects to the colliculus. This might account for the finding of limited visual capacities in the blind field of our subjects, despite the use of optimal stimuli. It might also explain why some subjects are more proficient than others in that the perturbed corticocollicular effects might not be exactly symmetric in all cases.

Notwithstanding the specific contribution of the superior colliculi and/or other structures to residual vision in the blind field, the present study has established that there are precise limitations for movement analysis. None of the experimental subjects succeeded in comparing the direction of grating movement in their blind field. These results are also concordant with those of Brindley *et al.* (1969) who found that their cortically blind patients could differentiate between sudden darkness or light onset but were unable to judge the direction of displacement of a drum. Blythe *et al.* (1987) also reported that movement detection mechanisms for residual vision in the blind field of their Case 25 was characterized by low directional selectivity. This failure is not surprising in view of the fact that tectal visual responses in the monkey are generally not specific to a particular direction of movement (Keating and Dineen, 1982), whereas recent evidence has shown that complex movement discrimination is probably mediated by cortical visual areas, such as MT and MST (Felleman and Van Essen, 1987; Rodman *et al.*, 1989). In fact, Perenin (1989) has reported that directional discrimination tasks could be carried out efficiently by striatectomized subjects but not by their hemispherectomized patient suggesting that this type of discrimination can only be achieved when the visual information is carried to a cortical area like MT on the side ipsilateral to the lesion.

The latter finding indicates that, in order to accomplish the detection and discrimination tasks used in the present study, our hemispherectomized patients did not rely heavily on the remaining hemisphere. Indeed, if this were the case, they should have shown some ability to discriminate direction since area MT on the opposite side was presumably intact. The question remains, however, as to how the interfield comparisons could be successfully carried out in some of our patients. We believe that this interaction between the two fields can be achieved at the midbrain level. Support for interfield integration occurring at a collicular level is provided by experiments on light difference thresholds. Singer *et al.* (1977) reported that the diminished capacity to detect repetitive light flashes in one half field could be reversed in normal subjects by stimulating a mirror-symmetric position in the contralateral visual field. This interaction between the two fields was also found in hemianopic patients when the mirror-symmetric position in the blind field was stimulated. Zihl and von Cramon (1979*a*) confirmed this finding by demonstrating that their patient with a congenital malformation of the right superior colliculus did not exhibit threshold elevation when stimulated repeatedly in the left visual field while stimulation in the right led to the expected increase in threshold. This was interpreted as suggesting that threshold elevation occurs as a consequence of collicular adaptation and that mirror-symmetrically organized interhemispheric interaction is mediated at collicular levels. A more recent study by Marzi *et al.* (1986) provided further evidence for an interaction between the two hemifields of hemianopic subjects. Most subjects demonstrated a tendency to react more quickly when two dots of light rather than one were flashed on either side of the vertical meridian even though they reported seeing only the stimulus presented in the intact field.

In essence, the results obtained here and in the studies described above indicate that there exists in the hemianopic visual field a series of residual visual capacities ranging from simple target detection to movement detection of gratings including more sophisticated interfield comparisons and discrimination of velocity differences. When the stimuli used meet the response properties of collicular cells (e.g., flashed targets, velocity and movement discrimination) hemispherectomized subjects show residual vision in their blind field. In the instance where the properties of the stimuli do not meet those of collicular cells (e.g., movement direction), the responses of the subjects remain at chance. It is therefore likely that in the total absence of the geniculostriate pathway in hemispherectomized subjects, the superior colliculi play an important role in mediating residual vision in the blind field. Finally, our results further indicate that in hemispherectomized subjects, both response accuracy in the blind field and access to conscious processes may vary according to the degree of correspondence between the tasks demands and collicular properties.

ACKNOWLEDGEMENTS

This work was supported by grants from the Natural Sciences and Engineering Research Council of Canada (NSERC) and from the Ministère de l'Education du Québec (Fonds FCAR) awarded to the authors. We wish to thank Dr Guy Geoffroy from the Hôpital Sainte-Justine de Montréal, for referring the patients.

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(Received October 30, 1989. Revised March 6, 1990. Accepted April 3, 1990)

Brain (1991), **114**, 513–525

SIMPLE AND CHOICE REACTION TIME PERFORMANCE FOLLOWING UNILATERAL STRIATAL DOPAMINE DEPLETION IN THE RAT

IMPAIRED MOTOR READINESS BUT PRESERVED RESPONSE PREPARATION

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SUMMARY

Rats were trained to perform a visual spatial discrimination, where stimulus luminance provided information regarding the required direction of response. The visual stimuli were presented either in advance of a temporally unpredictable auditory imperative stimulus (simple reaction time condition) or simultaneously with the imperative stimulus (choice reaction time condition). Following unilateral striatal dopamine depletion, produced by intracerebral infusion of 6-hydroxydopamine, there was not only a marked spatial response bias towards the side of the dopamine depletion but also an abolition of the delay-dependent speeding of reaction time that reflects motor readiness, on the side contralateral to the lesion. Nevertheless, the rats continued to show a benefit in performance of the simple reaction time condition compared with the choice reaction time condition, indicating that they were able to use advance information to select and prepare responses.

The results are discussed in the context of differential deficits shown by patients with Parkinson's disease in simple versus choice reaction time performance, and of the functions of parallel corticostriatal loops subserving the normal control of action.

INTRODUCTION

Some of the motor dysfunction in Parkinson's disease has been attributed to deficits in the advance construction or sequencing of a 'motor program' (Bloxham *et al.*, 1984, 1987) or the automatic execution of a learned motor plan (Marsden, 1982). This suggestion is partly based on evidence that patients with Parkinson's disease do not show lengthened reaction times in choice reaction time tasks, but they are impaired in simple reaction time performance, thus failing to show the same speeding of reaction time as healthy subjects when advance information is available (Flowers, 1978; Evarts *et al.*, 1981; Bloxham *et al.*, 1984, 1987; Sheridan *et al.*, 1987; Pullman *et al.*, 1988).

The neural basis for the seemingly paradoxical nature of the differential impairment of simple versus choice reaction time in Parkinson's disease is not well understood, although it presumably has implications for understanding the neural substrates of normal, as well as abnormal, action. Thus it is unclear to what extent the deficit in simple reaction time is either dopaminergic or indeed striatal in nature. This is an important consideration,

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given that parkinsonian patients exhibit neurochemical deficits in noradrenergic, serotonergic and cholinergic innervations of the neocortex (Scatton *et al.*, 1982; Agid *et al.*, 1987) as well as dopaminergic depletion in prefrontal cortex (Scatton *et al.*, 1982). Furthermore, a significant relationship has been found between simple reaction time performance and CSF levels of the noradrenergic metabolite, 3-methoxy-4-hydroxyphenylglycol, in parkinsonian patients (Stern *et al.*, 1984).

Pullman *et al.* (1988) have recently attempted to resolve the neurochemical nature of the reaction time deficit by studying the effects of levodopa treatment on simple and choice reaction time tasks in patients with Parkinson's disease. They concluded that, whereas choice reaction time performance was improved with treatment, simple reaction time was not (*see also* Rafal *et al.*, 1984). On this basis, they further concluded that simple reaction time paradigms may be at least partially dependent on 'non-dopaminergic physiologic mechanisms' and, in particular, upon the integrity of the supplementary motor area (SMA) and its noradrenergic connections, whereas choice reaction time depends on components of a 'functional loop' comprising a caudatothalamoprefrontal cortical pathway (*cf.* Alexander *et al.*, 1986) the function of which is modulated by striatal dopamine.

However, there are problems in inferring the neural substrate of reaction time performance from the data of patients with Parkinson's disease in this way. First, it is clear that the SMA also forms a functional loop with the striatum which is similarly modulated by dopamine, in particular within the putamen (Alexander *et al.*, 1986). Secondly, the latter striatal region exhibits the greatest degree of dopamine depletion in Parkinson's disease (Hornykiewicz, 1982; Garnett *et al.*, 1984). Thus according to the Pullman *et al.* (1988) scheme, it is difficult to understand why simple reaction time performance should not depend on striatal dopamine.

With these problems of interpretation in mind, it seems likely that neither the neural basis of reaction time deficits in Parkinson's disease nor the role of striatal dopamine in simple and choice reaction time tasks will be easily resolved from studies of human patients. The present experiment therefore examined the effects of selective depletion of striatal dopamine (using the neurotoxin, 6-hydroxydopamine (6-OHDA)) on performance in a paradigm allowing measurement of both simple and choice reaction time in the rat. A unilateral preparation was used which enabled each animal to serve as its own control and avoided the debilitating consequences of a bilateral lesion (Carli *et al.*, 1989). Previous experiments have examined the effects of unilateral striatal dopamine depletion in the rat in reaction time paradigms (Carli *et al.*, 1985, 1989; Brown and Robbins, 1989*a*). In the former two studies, choice reaction time procedures that varied in the degree of stimulus-response compatibility were used, and in the third study simple and choice reaction time were compared directly. In all three studies, unilateral striatal dopamine depletion did not differentially affect reaction time performance, slowing occurring in all cases for responses contralateral to the side of the lesion. However, in none of these experiments has the effect on precueing, and hence advance information, been specifically investigated. The issue of possible differential effects of Parkinson's disease on simple and choice reaction time, resulting from a failure to use advance information efficiently, may be of central importance for understanding the nature of the parkinsonian deficit. Thus the present experiment was designed to examine whether striatal dopamine depletion, per se, produced a differential impairment in the ability to use advance information to prepare a response in simple versus choice reaction time procedures.

METHODS

Subjects

The subjects were 9 male Lister Hooded rats (Olac, Bicester), weighing 200–250 g at the beginning of the experiment. They were housed in pairs in natural lighting and maintained at 90% of their free-feeding body weight throughout the experiment on 15 g per day of standard laboratory chow, provided after the daily experimental session.

Apparatus and procedure

The apparatus used has been described in detail previously (*see* fig. 1, Carli *et al.*, 1989; Brown and Robbins, 1989*a*). The operant chamber had an array of response holes, monitored by photoelectric cells, with a stimulus light at the rear of each hole. Three holes were open to the animal; the animal was required to hold its nose still in the central of the three holes, whilst the stimuli were presented in the adjacent side holes. Each trial was initiated by the animal, by making a response in the central hole. The light in the central hole was illuminated until the animal made such a response and was extinguished to signal that the trial had begun.

The rats performed a brightness discrimination, in which the stimuli were not spatially localized, but presented to both sides of the animal's head. Bright stimuli signalled that a response in the left side hole was correct, whilst dim stimuli signalled that a response in the right side hole was correct.

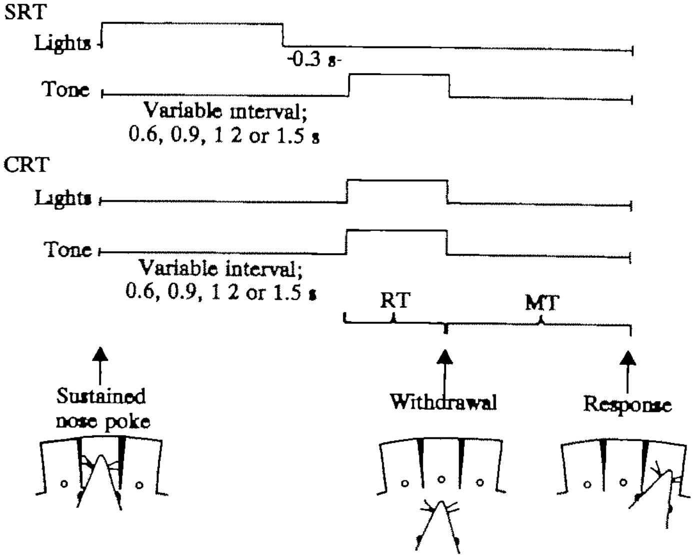

FIG 1. Task requirements of the simple (SRT) and choice (CRT) reaction time tasks The upper lines show the simple reaction time condition in which the lights signifying the direction of the required response (either bright, for a left response, or dim, for a right response) were presented at the beginning of a trial. The lights were extinguished 0.3 s before the presentation of the auditory imperative signal. The choice reaction time condition is represented in the lower lines. The informative lights and the imperative tone were presented simultaneously and both remained on until a response was initiated

In order to compare the effects of advance information, or simple reaction time, with no advance information, or choice reaction time, a tone was used in addition to the visual stimuli. The tone was the imperative signal and occurred after an unpredictable delay, staying on until the animal responded by withdrawing its nose from the central hole. The delay before the tone was 0.6, 0.9, 1.2 or 1.5 s.

The lights specifying direction of responses were presented either simultaneously with the tone (choice reaction time condition) or presented as soon as the rat responded in the central hole, at the beginning of the variable delay, and were extinguished 0.3 s before the tone (simple reaction time condition). These task requirements are shown in fig. 1.

In any one session, all the trials were either the simple or the choice condition. The rats were tested for 8 consecutive days, in the simple and choice reaction time conditions on alternate days, to obtain the preoperative baseline values, before receiving 6-OHDA lesions. Five rats were lesioned on the right and 4 rats were lesioned on the left After a period of postoperative recovery lasting 4 days, they were tested for 8 consecutive days, again alternating the simple and choice reaction time conditions. They were tested again 30 and 60 days postoperatively, to examine the extent of recovery.

Design and analysis

The same rats performed both the simple and the choice variants of the reaction time task. Unilateral, rather than bilateral lesions were employed in order to compare performance on the lesioned and the unlesioned side in the same animal.

The measures used were: percentage correct; time to initiate responses (reaction time) from the onset of the stimuli to the withdrawal of the head from the central hole; time to complete responses (movement time) from the withdrawal of the head from the central hole to the response in the side hole; spatial response bias (bias), a measure calculated as number of responses to the ipsilateral side divided by total number of responses made; frequency of responses made during periods of timeout, following incorrect or premature responses.

Data were analysed by analysis of covariance (ANCOVA), with preoperative baseline values used as the covariate. The rationale for this was to test for a significant departure in the pattern of postoperative data compared with the preoperative pattern. Thus where the effects were not significant in the ANCOVA they could be assumed not to differ from the preoperative state, whilst significant effects indicated a departure in the pattern of data from the preoperative baseline. Polynomial regression analysis was performed on the reaction time data to test the significance of linear and other components, especially of the delay dependent effects. Significant interactions were further analysed by post-hoc Neuman-Keuls comparisons. Transformations were applied to percentage and reaction time data (angular and $\log_{10}$, respectively) to normalize the distributions in accordance with the ANOVA model (Winer, 1971).

Surgery

Following training to stable performance, the rats received unilateral lesions of the striatum by intrastriatal infusion of the neurotoxin 6-hydroxydopamine (6-OHDA).

The rats were treated with 50 mg/kg of pargyline hydrochloride (Sigma Chemical Co.) dissolved in 0.9% sterile saline, injected i.p. 30 min before surgery, to enhance the effect of 6-OHDA (Breese and Traylor, 1971). Surgery was performed under Equithesin anaesthesia (0.3 ml/100 g, i.p.) in clean conditions. The animal was placed in a Kopf stereotaxic instrument, fitted with atraumatic earbars, and injected unilaterally with 6-OHDA (6-OHDA hydrobromide, Sigma Chemical Co.) freshly dissolved in 0.1 mg/ml ascorbic acid in 0.9% saline; 2 μl of vehicle, containing 8 μg of 6-OHDA (base), were infused over 4 min via a 30-gauge stainless steel cannula, attached to a Harvard infusion pump with PP10 polythene tubing. The cannula was left in place for 2 min before and after the infusion. The coordinates used were 2.0 mm anterior to the bregma, ±3.5 mm from the midline and −5.5 mm from the cortical surface.

Neurochemical assay

Six weeks after surgery, the rats were killed under ether anaesthesia, the brains rapidly removed, and the tissue dissected on ice as previously described (Carli *et al.*, 1989) into the nucleus accumbens and the anterior caudate-putamen (taken from the same 2 mm thick slice). The posterior caudate-putamen and the tail of caudate were taken from the next 2 posterior 2 mm slices, respectively. The tissue was stored at −70° C until assay.

Tissue concentrations of dopamine were measured by high performance liquid chromatography with electrochemical detection (HPLC-EC), based on the method described by Mefford (1981) and described in detail in Carli *et al.* (1989).

RESULTS

Neurochemical results

The Table shows the results of the neurochemical assay of DA concentrations. There was profound (>85%) depletion of dopamine throughout the anterior, posterior and tail portions of the caudate-putamen, with mean depletions of less than 50% in the nucleus accumbens.

TABLE. MEAN AND SEM OF CONCENTRATIONS OF DOPAMINE IN THE STRIATUM EXPRESSED AS ng/mg OF WET TISSUE, IPSILATERAL AND CONTRALATERAL TO THE SIDE OF INFUSION OF THE NEUROTOXIN, 6-OHDA, AND MEAN AND SEM OF PERCENTAGE DEPLETIONS OF DOPAMINE IN THE CONTRALATERAL STRIATUM

	Concentrations		
Area	*Ipsi*	*Contra*	*Mean % depletion*
Anterior caudate	1.60 (1.3)	13.50 (1.6)	87.8 (10.5)
Posterior caudate	1.10 (1.0)	13 70 (2.7)	91.1 (10.0)
Tail of caudate	1.10 (1.5)	8.40 (3.7)	84.6 (18.4)
Nucleus accumbens	4.65 (2 3)	8.50 (1.7)	48.8 (25.5)

Behavioural results

Reaction time performance. Fig. 2 shows simple and choice reaction time, preoperatively and postoperatively, of responses to each side, plotted as a function of delay. Reaction times decrease as a function of delay preoperatively (F(3,21) = 26.14, $P < 0.001$), with faster reaction times at the longer delays due to the increasing probability of the occurrence of the stimulus as the delay elapses. This result was confirmed by finding a highly significant linear regression component (F(1,21) = 75.54, $P < 0.01$).

In addition to the delay-dependent speeding of reaction time, reaction time was also faster in the simple, as compared with the choice reaction time condition. This was evident as a Condition by Delay interaction (F(3,21) = 4.81, $P = 0.011$) which, when further analysed by post hoc Newman-Keuls comparisons, was found to result from there being a significant benefit to reaction time of the simple over choice reaction time condition at the two longest delays (1.2 and 1.5 s). There was a near significant difference at the delay of 0.9 s. Only at the shortest delay was there no significant difference between simple and choice reaction time. The Condition by Delay interaction had a highly significant linear (F(1,21) = 11.48, $P = 0.003$) but not quadratic (F(1,21) = 2.95, $P = 0.10$) or other (F(1,21) = 1.0, n.s.) component indicating that the two conditions mainly differed in the slope of the linear regression. The faster reaction time in the simple, as compared with the choice reaction time condition and the effect of delay remained unchanged postoperatively (F(3,21) = 0.75, n.s.). In particular, the crucial

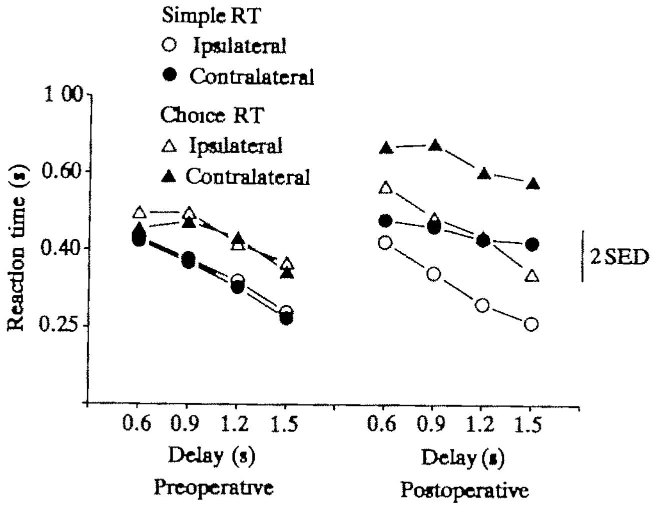

FIG. 2. Reaction time of responses to the ipsilateral and contralateral side in the simple and choice reaction time conditions, plotted as a function of delay. Simple reaction time (circles) was faster than choice reaction time (triangles) both preoperatively and postoperatively. Reaction time decreased as a linear function of delay preoperatively in both simple and choice conditions and on both sides. This effect was abolished on the side contralateral to the infusion of 6-OHDA (filled symbols). The vertical bar shows two standard errors of the difference of the means (2 SEDs) associated with the interaction between Surgery, Side and Delay.

comparison, the postoperative interaction between Condition (simple versus choice reaction time) and Side of response (ipsilateral or contralateral to the lesion), which would have indicated a differential impairment in either simple or choice reaction time on the lesioned side, was not significant (F(1,7) = 0.98, n.s.).

Independent of the advantage of simple over choice reaction time at the longer delays, postoperatively there was an interaction between the factors of Side of response and Delay (F(3,19) = 3.64, P = 0.03). Post hoc Newman-Keuls comparisons revealed that at the shortest delay, ipsilateral and contralateral reaction times were not different from each other. At the delay of 0.9 s, the difference approached significance and at the longer delays of 1.2 and 1.5 s, contralateral reaction time was significantly slower than ipsilateral reaction time. From fig. 2 it is clear that whilst ipsilateral reaction time continues to show a linear reduction as delay elapses, contralateral reaction time no longer shows the effect of the increasing probability of the stimulus. Post hoc Newman-Keuls comparisons revealed that ipsilateral reaction time was significantly faster at the longer delays of 1.2 and 1.5 s, compared with the 0.6 s delay, but contralateral reaction time was not significantly different at delays of 0.6 and 1.5 s. Polynomial regression analysis confirmed a significant linear (but no other) component for the Side by Delay interaction (F(1,19) = 9.21, P = 0.007).

There was no significant difference between the reaction times of correct and incorrect responses (F(1,8) = 0.36, P = 0.57). A significant Side of response (ipsilateral or contralateral) by Response Outcome (correct versus incorrect) interaction (F(1,8) = 6.57, P = 0.037) was due to a difference in reaction time between ipsilateral and contralateral correct responses (0.32 as compared with 0.47 s, respectively), whereas no such difference was found for incorrect responses, which fell midway between the two and did not differ between sides (ipsilateral, 0.39 s and contralateral, 0.43 s).

Response accuracy. Fig. 3 shows the percentage correct of responses made in the simple and the choice conditions preoperatively and postoperatively. There was a greater accuracy of performance in the simple reaction time condition which was confirmed by a significant main effect of Condition preoperatively (F(1,7) = 42.3, $P < 0.001$). This effect did not change postoperatively (F(1,6) = 0.55, n.s.); simple reaction time performance remained more accurate than choice reaction time performance, further demonstrating that the rats continued to use the advance information in performing this task.

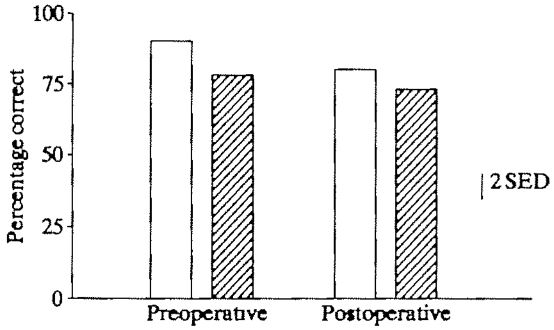

FIG. 3. Percentage correct in the simple (open bars) and choice reaction (hatched bars) time conditions is shown preoperatively and postoperatively; there was a higher percentage correct in the simple reaction time condition which was not differentially affected by unilateral striatal dopamine depletion The bar shows two standard errors of the difference of the means associated with the factor Condition (simple vs choice reaction time)

Responses to the dim stimuli were less accurate than responses to the bright stimuli (F(1,7) = 8.77, $P = 0.02$). There was an average of 74.6% correct to dim stimuli and 78.6% correct to the bright stimuli. Once again, this effect was not changed postoperatively (F(1,6) = 0.03, n.s.).

Response bias. Fig. 4 shows response bias for the simple and choice reaction time conditions postoperatively, plotted as a function of delay. From no bias (50%) preoperatively, bias towards the side of the lesion was increased to 74% postoperatively. The degree of spatial response bias was the same for simple and choice reaction time performance (F(1,8) = 0.06, n.s.) and bias also did not vary as a function of delay (F(3,24) = 0.74, n.s.), indicating that the apparent greater impairment in reaction time at the longer delays was not an effect of greater response bias, nor did it result in greater bias.

Bias during timeout periods. Postoperatively, fewer responses were made in the hole contralateral to the side of dopamine depletion (mean of 17, compared with 38.5 ipsilateral responses), the significance of which was confirmed by the interaction between the factors of side of Lesion and Side of response (F(1,6) = 14.75, $P = 0.009$).

Movement time. The average of 0.18 s for movement time preoperatively, was nonsignificantly increased to 0.20 s postoperatively. There was a main effect of Condition

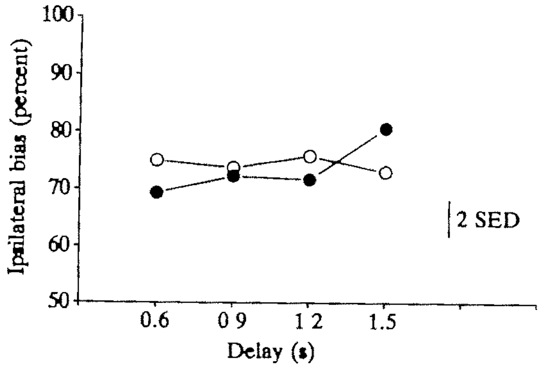

FIG 4. Response bias to the ipsilateral side is plotted as a function of delay before the imperative signal Postoperatively, animals were biased to the ipsilateral side in both the simple (open circles) and the choice (filled circles) reaction time conditions and bias did not change significantly as a function of condition or delay The bar shows two standard errors of the difference of the means associated with the interaction between Condition and Delay.

(F(1,6) = 9.38, P = 0.02) which was due to faster movement times in the simple reaction time condition (0.18 compared with 0.22 s), but this did not change after surgery. There was no effect of side of lesion on movement time (F(1,6) = 0.11, n.s.).

Lack of behavioural recovery. The rats were tested again 30 and 60 days after surgery for 8 consecutive days. The impairments were still apparent up to 60 days postoperatively. Bias was not significantly reduced compared with the first postoperative test (F(1,7) = 1.59, n.s.); immediately postoperatively, bias was 73.7% and after 60 days it was still 69.1%. The reaction time effects also remained unchanged over the three postoperative tests (F(2,14) = 1.39, n.s.).

DISCUSSION

The results of the present experiment have clearly demonstrated that rats with profound dopamine loss in the striatum on one side are able to use advance information in the selection and preparation of responses to either side, and continue to show the same magnitude of speeding of reaction times and increases in performance accuracy when provided with advance information, while exhibiting specific deficits in reaction time performance.

The present behavioural deficits arise from profound (up to 90%), and rather selective, depletion of dopamine from the caudate-putamen. Although 6-OHDA destroys noradrenergic as well as dopaminergic neurons, these neurons represent only a minor portion of striatal catecholaminergic innervation (McGeer *et al.*, 1984) and therefore depletion of noradrenaline probably does not account for the behavioural impairments reported here. Moreover, substantially greater depletion of dopamine unilaterally from the nucleus accumbens, larger than reported here, has been shown to be without effect on rotational behaviour (Kelly and Moore, 1976). The behavioural impairments were long-lasting, persisting for at least 2 months postoperatively, thus contrasting with the many reports of only transient deficits in spontaneous behaviour following striatal dopamine depletion (e.g., Ungerstedt, 1971; Marshall, 1979).

Motor programming: comparison of simple and choice reaction time performance

The performance enhancements provided by advance information in the simple, as distinct from the choice, reaction time condition remained both for responses ipsilateral *and* contralateral to the side of dopamine depletion. These results have important implications for understanding not only the apparently selective deficit in simple reaction time seen in Parkinson's disease that was described in the Introduction, but also the neuropsychological substrates of action. The failure to find differential effects of striatal dopamine depletion on performance of these tasks extends the previous findings of no differential impairment in simple reaction time (Brown and Robbins, 1989*a*) to situations where the response is precued. Although our results may appear to contrast with some of those seen previously in patients with Parkinson's disease (e.g., Bloxham *et al.*, 1987; Pullman *et al.*, 1988), they are not necessarily inconsistent with the clinical findings. First, from the results of Pullman *et al.* (1988), it appears that L-dopa medication, in fact masks a deficit in choice reaction time in parkinsonian patients. Nevertheless, simple reaction time performance appears to be relatively impervious to L-dopa medication and this may yet indicate that simple and choice reaction time performance are mediated by distinct neural substrates. However, this argument may be fallacious because simple and choice reaction time performance may provide control baselines which are differentially sensitive to L-dopa medication because of scaling factors. Secondly, in the Pullman *et al.* (1988) study, it is clear that there is a trend for simple reaction time also to show improvement after L-dopa medication, which may have been significant had a larger sample of patients (than the 5 tested) been used. Therefore it may not be surprising that our results show no differential effect on simple and choice reaction time performance in rats with striatal dopamine depletion.

In a group of parkinsonian patients that perhaps is of greatest relevance to the present experiment, namely largely unmedicated patients with hemiparkinsonism, Rafal *et al.* (1989) showed that the simple reaction time was faster than choice reaction time when tested using the 'bad' hand, showing clear evidence of clinical signs of parkinsonism (presumably contralateral to the side of greatest dopamine depletion) as well as when tested with the asymptomatic ('good') hand.

In neuropsychological terms, the present results have failed to support the implication that the speeding of reaction time in simple reaction time tasks is not dependent on striatal dopamine (Pullman *et al.*, 1988) but rather suggest that both simple and choice reaction time performance are striatally influenced. This is not to imply that we think that simple and choice reaction time could not be mediated by different neural substrates within the striatum, for example, by different segregated and parallel corticostriatal loops (Alexander *et al.*, 1986), although the results do not provide direct evidence for this possibility.

Motor readiness

Although the reaction time advantage of the provision of advance information remained for contralateral responses, unilateral striatal dopamine depletion did impair contralateral responding, in agreement with earlier results (Carli *et al.*, 1985, 1989; Brown and Robbins, 1989*a*; Robbins and Brown, 1990). Fewer responses were initiated to the contralateral side, with approximately 75% of all responses being directed ipsilaterally. This enhanced spatial bias has been argued to represent alterations in response set, the prior assignment of probability of selection from the repertoire of available responses (Brown and Robbins, 1989*b*; Carli *et al.*, 1989). Contralateral reaction times were lengthened postoperatively, relative to ipsilateral reaction times, but as a function of stimulus presentation delay, with significant differences between the two sides only at the longest delays. This result appears different from that seen in an earlier study (Carli *et al.*, 1989) which also showed delay-dependent changes in reaction time, but with differences at the shorter rather than the longer intervals. However, that study used a different paradigm for measuring reaction time in which the stimuli were presented only to one side of the animal's head at a time, rather than bilaterally. The previous results were consistent with an explanation in terms of lateralized attentional strategies resulting from exaggerated spatial response biases towards the side of the lesion (*see* Carli *et al.*, 1989). By employing a bilateral mode of stimulus presentation, the present study prevented the development of such lateralized attentional strategies which would have contaminated the reaction time measures used here.

We use the term 'motor readiness' to refer to the speeding of reaction time of responses as the delay elapses. In both the simple and choice reaction time conditions, the trials are initiated by the rat itself with a centre hole response. This response extinguishes the light in the central hole which marks the start of the delay. At the start of the delay, the rat cannot know for how long it must wait, but as the delay elapses the occurrence of the stimulus becomes increasingly likely. Thus the speeding of reaction time results from the gradual increase, as delay elapses, in the conditional probability of the occurrence of the visual stimulus (until certainty is reached at the longest delay, 1.5 s). The delay-dependent speeding of reaction time has been reported previously in human reaction time studies (Näätänen and Merisalo, 1977; Frith and Done, 1986) where it appears to reflect some aspect of response preparation. In agreement with Frith and Done (1986), it was found in the present study that choice, as well as simple, reaction time showed a linear speeding as a function of delay. This strongly suggests that the effect does not arise from the preparation of a specific response, but rather from a more general readiness to respond.

The attenuation of the delay-dependent speeding of reaction time of responses contralateral to the side of dopamine loss implies that this general readiness to respond is mediated at least in part by striatal dopamine. Furthermore, as it is only responses contralateral to the side of dopamine loss which show a failure to benefit from increasing stimulus probability, it appears that 'motor readiness' is lateralized and does not result from generalized bilateral activation.

As stimuli of different intensities were used, the present experimental design also allowed a specific test to be made of the dopaminergic mediation of the nonspecific activation that can arise from the effects of stimulus intensity (*see* Posner, 1978, p. 113). Responses were more accurate to bright than to dim stimuli, and this effect was preserved postoperatively for both ipsilateral and contralateral responses, showing that there was still an effect of nonspecific activation following the reduction in striatal dopamine. Previously, it has also been shown that irrelevant white noise presented just before, or simultaneously with, a visual stimulus speeded to the same degree responses both ipsilateral and contralateral to the side of striatal dopamine depletion (M. Carli, T. W. Robbins, J. L. Evenden, unpublished observations; *see* Robbins and Brown, 1990), reminiscent of a similar finding in patients with Parkinson's disease (Heilman *et al.*, 1976), and indicating that this generalized activation by an external stimulus is not mediated by striatal dopamine.

The dual effects of impaired 'readiness' of contralateral responses and enhanced ipsilateral response bias do not necessarily reflect a common deficit, for example, of response set. It this were true, then it might be expected that, as the ipsilateral responses become increasingly primed by delay, bias to the ipsilateral side would also increase. In fact, no such effect was seen; bias was equal at all stimulus delays. This dissociation of bias and reaction time has also been observed by us in a different paradigm in which the two measures were differentially affected by cell body lesions of the lateral and medial striatum, respectively (Brown and Robbins, 1989*b*). Thus it is clear that bias is neither a product of reaction time nor the cause of the reaction time effects, and bias and reaction time measures are functionally dissociable, although the same general deficit might underlie each.

In summary, unilateral striatal dopamine depletion appears to result in lateralized changes in motor readiness generated by endogenous cues that are unrelated to motor programming per se. This is especially relevant to apparently similar findings in patients with Parkinson's disease by Bloxham *et al.* (1987) of a significant interaction between the foreperiod and reaction time, with reaction time decreasing as a function of delay in the control group but not in the parkinsonian patients. This deficit in the internal generation of motor readiness may reflect the well-known differential susceptibility of patients with Parkinson's disease to deficits when movements are initiated voluntarily, rather than in response to external cues (e.g., Martin, 1967; Flowers, 1976, 1978; Cooke *et al.*, 1978; Stern *et al.*, 1980). It may also correspond to the impairments in shifting cognitive set following internally derived, rather than externally provided, information (Brown and Marsden, 1988). The effects of striatal dopamine depletion on motor readiness are of particular interest as this is one of the functions attributed to the SMA, one component of a loop involving the basal ganglia and ventrolateral thalamus (Goldberg, 1985). Activation of this loop gives rise to the Bereitschaftspotential that is associated with the preparation of voluntary movement and which Deecke (1985) has described as having a 'priming function for our freely voluntary, fully endogenous movements and actions'. The Bereitschaftspotential, has recently been reported to be reduced both in duration and amplitude in cases of Parkinson's disease (e.g., Simpson and Khuraibet, 1987; Dick *et al.*, 1989). These considerations and the results reported here on the effects of striatal dopamine depletion in the rat suggest that it would be of interest to examine the effects of temporal predictability and stimulus uncertainty in Parkinson's disease.

ACKNOWLEDGEMENTS

This work was supported by a Major Award from the Wellcome Trust. V.J.B. was in receipt of a Medical Research Council studentship. We would like to thank Dr R. D. Rafal for helpful comments on the manuscript.

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(*Received August 11, 1989. Revised March 6, 1990. Accepted April 4, 1990*)

Brain (1991), **114**, 527–540

PARAESTHESIAE AND TETANY INDUCED BY VOLUNTARY HYPERVENTILATION

INCREASED EXCITABILITY OF HUMAN CUTANEOUS AND MOTOR AXONS

by GARY MACEFIELD *and* DAVID BURKE

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SUMMARY

Anxiety can induce hyperventilation, and the resultant hypocapnia and hypocalcaemia can lead to paraesthesiae and tetany. To define the nature of the disturbance created in peripheral nerve, the excitability of cutaneous and motor axons was monitored in 6 normal subjects requested to hyperventilate until paraesthesiae developed in the hands, face and trunk. This occurred when alveolar PCO_2 (P_ACO_2) had declined on average by 20 mmHg. Spontaneous EMG activity developed when P_ACO_2 had declined by a further 4 mmHg. Changes in the excitability of cutaneous and motor axons were measured from changes in the compound action potentials evoked by stimulation of the median nerve at the wrist and recorded over the digital nerves of the index finger and over the thenar muscles, respectively. As P_ACO_2 declined, the size of the compound sensory and muscle potentials evoked by a constant stimulus progressively increased, indicating an increase in axonal excitability. These changes occurred before paraesthesiae or tetany developed. In each subject there was a statistically significant inverse correlation between P_ACO_2 and axonal excitability. Independent of this increase in axonal excitability, there was no significant change in the supernormal phase of the recovery cycle of cutaneous axons. Microneurographic recordings from the median nerve in 2 subjects revealed spontaneous bursting activity of cutaneous axons, perceived as paraesthesiae. It is concluded that the paraesthesiae and tetany induced by hyperventilation result solely from changes in the excitability of cutaneous and motor axons in the peripheral nerve, presumably due to an alteration in the electrical properties of the axonal membrane resulting from a reduced plasma [Ca^{2+}]. The supernormal phase may entrain the ectopic discharge and thereby determine the maximal discharge frequency of impulses in ectopically generated trains, but does not otherwise contribute to the physiological disturbance.

INTRODUCTION

During an attack of anxiety, breathing may be stimulated to such an extent that CO_2, the primary respiratory stimulus, is blown off and the hyperventilation maintained by the emotional disturbance itself. Neurological consequences of the hypocapnia incude the development of paraesthesiae in the face, trunk and distal extremities and fasciculation or tetanic spasm of muscles (Kugelberg, 1948; Fanconi and Rose, 1958; Davis *et al.*, 1970). In clinical practice, the 'hyperventilation syndrome' is one of the commonest causes of distal symmetric paraesthesiae, perhaps even more common than polyneuropathy. In many peripheral nerve disturbances paraesthesiae result from the high-frequency

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ectopic discharge of cutaneous axons (Ochoa and Torebjörk, 1980; Nyström and Hagbarth, 1981; Culp *et al.*, 1982; Nordin *et al.*, 1984; Burke and Applegate, 1989). Similarly, spontaneously active motor axons are responsible for fasciculation and tetany (Kugelberg, 1948; Bergmans, 1982).

The respiratory alkalosis associated with hyperventilation causes a reduction in plasma $[Ca^{2+}]$ (Fanconi and Rose, 1958; Davis *et al.*, 1970) and thereby increases the axonal excitability of peripheral nerves, leading to the generation of repetitive discharges (Katz, 1936; Lehmann, 1937*b*; Brink *et al.*, 1946; Frankenhaeuser, 1957; Frankenhaeuser and Hodgkin, 1957; Hille, 1968). Although an increase in extracellular pH can increase excitability, this is considered to act via the change in $[Ca^{2+}]_o$ (Lehmann, 1937*a*). Paraesthesiae may also be produced by the intravenous infusion of hypocalcaemic agents (Davis *et al.*, 1970; Becker *et al.*, 1974). Hyperventilation and artificially induced hypocalcaemia have been shown to ameliorate the impaired visual function associated with multiple sclerosis, presumably by improving impulse propagation in demyelinated axons (Davis *et al.*, 1970; Becker *et al.*, 1974; Davies *et al.*, 1986).

The purpose of the present study was to extend earlier electromyographic observations on hyperventilation-induced tetany in human subjects (Kugelberg, 1948) and, in particular, to investigate changes in cutaneous axons that could account for the generation of ectopic impulses and the consequent development of paraesthesiae. These changes were correlated with alveolar PCO_2. Microneurographic recordings were also made from spontaneously discharging cutaneous afferents in the median nerve.

METHODS

Twelve experiments were performed on 4 male and 2 female subjects (age 20–44 yrs), each of whom was neurologically normal and gave informed consent. Additional microneurographic studies were performed on the 2 authors. All experiments were conducted with the approval of the institutional ethics committee.

The protocol consisted of monitoring the electrical excitabilities of cutaneous and motor axons of the median nerve during voluntary hyperventilation. Subjects were supine and, after control measurements were made, were requested to breath deeply but slowly until intense paraesthesiae of the hands, face and trunk developed. Changes in cutaneous axon excitability were measured from changes in the antidromically-conducted compound sensory action potential evoked by stimulation of the median nerve at the wrist and recorded over the digital nerves of the index finger, or the orthodromically-conducted potential evoked by stimulation of the digital nerves and recorded over the nerve. In the latter paradigm, changes in the supernormal period were also measured. Changes in motor axon excitability were indicated by changes in the size of the compound muscle action potential evoked by stimulation of the median nerve and recorded over the thenar muscles. Skin temperature was monitored continuously in all experiments at the base of the digit and at the wrist, and was maintained between 35° C and 36° C by radiant heat and blankets.

Stimulation procedures

In the first series of 6 experiments rectangular pulses of 50 μs duration were delivered at 2 Hz from a constant-current source (MS20, Medelec) to the median nerve at the wrist, using surface electrodes with the anode on the dorsum. This allowed the antidromically-conducted compound sensory action potential and the compound muscle action potential to be recorded simultaneously. In each subject a stimulus-response relationship was established between current and the size of the sensory and muscle action potentials. A stimulus level producing a motor potential usually ~40% of its maximal size was set as the test stimulus for assessing changes in excitability of cutaneous and motor axons during hyperventilation.

In the second series of 6 experiments the stimuli were delivered at 1 Hz to the digital nerves of the index finger via ring electrodes around the proximal and middle phalanges. In addition to establishing the relationship between current and amplitude of the compound sensory action potential, the refractory and supernormal phases of the recovery cycle of cutaneous axons were determined, using a test volley of approximately 30% of maximum preceded by a maximal conditioning volley at intervals of 1.5–26.0 ms (Ng *et al.*, 1987; Applegate and Burke, 1989). The conditioning and test stimuli were delivered from separate constant-current sources. The conditioning-test interval producing maximal supernormality was determined for each subject. During hyperventilation, changes in supernormality were assessed using this conditioning-test interval. In addition, because the degree of potentiation produced by supernormality varies inversely with the size of the unconditioned test volley (Ng *et al.*, 1987; Applegate and Burke, 1989; *see* fig. 8A), the degree of supernormality was determined for a range of test stimulus intensities, before and during hyperventilation, when alveolar PCO_2 had reached a steady state. Changes in excitability were measured from changes in the amplitude of the *unconditioned* test volley.

Recording procedures

In the first series the antidromic sensory potential was recorded via ring electrodes around the index finger, simultaneously with the compound muscle potential recorded via surface electrodes over the thenar muscles. The signals were amplified (5×10^4 and 5×10^2, respectively), filtered (10 Hz–2 kHz) and the 15 ms poststimulus period digitized at 0.07 MHz (MS20, Medelec). Averages of 32 sweeps each were stored on disc at 1 min intervals during the hyperventilation, commencing after 1 min. After 9 min, at which time alveolar PCO_2(P_ACO_2) had attained a steady state, the intensity of the test stimulus was varied to allow determination of the stimulus-response relationship.

In the second series the orthodromic sensory action potential was recorded (1×10^5, 10 Hz–2 kHz) over the median nerve at the wrist using surface electrodes. Sequences in which the test volley was preceded by the maximal conditioning stimulus and those in which it was not were stored as separate averages of 32 sweeps each. These pairs of averages were recorded at 1.5 min intervals, commencing 0.5 min after the commencement of hyperventilation. At each interval, the unconditioned test volley served as an indication of axonal excitability and provided the control test volley for the conditioned test potential.

In all experiments end-tidal PCO_2 was recorded continuously via an oral catheter (diameter 1 mm), on the basis of its infra-red absorption (Normocap, Datex), and displayed on a chart recorder. Subjects visually monitored their peak PCO_2. Alveolar PCO_2 was recorded intermittently by requesting the subject to make a maximal slow exhalation. This was done before and after each averaging sequence as hypocapnia was developing, and at regular intervals when the subjects maintained a steady level of hypocapnia.

In additional experiments microneurographic techniques (*see* Vallbo *et al.*, 1979) were used on the authors to record directly from cutaneous axons during hyperventilation-induced paraesthesiae. The subject was seated and the forearm and hand supported on a padded table. An insulated tungsten microelectrode was inserted percutaneously into the median nerve at the wrist, and manually directed into a fascicle supplying the skin of a digit whilst delivering electrical stimuli (1–10 V, 0.1 ms, 1 Hz; Grass S88 and SIU5) through the microelectrode. Neural activity was amplified (2×10^4), filtered (0.3–3.0 kHz) and stored on magnetic tape with P_ACO_2 and voice signals. Once a suitable site had been secured the subject started to hyperventilate, reporting the development of paraesthesiae verbally on tape. To ensure that the recorded neural activity was not contaminated by EMG activity from adjacent muscles, the neurogram was monitored on a loudspeaker using an open bandwidth during the experiment and again during replay of the recorded data, prior to further filtering (high pass 700 Hz) and integration (*see below*).

Analysis

Analyses of compound sensory and motor action potentials were made from retrieved information stored on the MS20, which provides cursor-based measurements of latency, amplitude and area. Changes in the size of the antidromic sensory potential were calculated on the basis of area because this potential lacks an initial positivity (fig. 3A). The total area of the negative and positive phases of the potential was determined by measuring from the onset of the negativity to the end of the 10 ms sweep; the compound muscle potential was measured likewise. The amplitude of the orthodromically-conducted sensory potential, recorded at the wrist, was measured from the initial positive peak to the subsequent negative peak. All values were normalized to the mean of 3 control measurements. Microneurographically recorded activity was replayed through an integrator (time constant 100–200 ms) and displayed on an electrostatic recorder. Data were evaluated statistically using analysis of variance, paired t tests or the Mann-Whitney U test (StatWorks 1.2a, Cricket Software).

RESULTS

Spontaneous activity of cutaneous axons and motor units during hyperventilation

All subjects were instructed to hyperventilate slowly until paraesthesiae and mild tetany developed, at 1–10 min and 6–19 min, respectively. Alveolar PCO_2 was measured at the end of a full exhalation. Paraesthesiae of the face or hand did not develop until P_ACO_2 had fallen by 10–29 mmHg (mean ± SD, 20 ± 6 mmHg); in each subject, twitching of facial muscles or of the hand occurred when P_ACO_2 had declined further, by 2–5 mmHg (4 ± 1 mmHg). In each subject the levels of hypocapnia at which paraesthesiae and twitching occurred were fairly consistent when tests were repeated on different days.

In 2 subjects, microneurographic recordings of cutaneous afferent activity were made from the median nerve, simultaneously with thenar electromyographic (EMG) activity recorded via surface electrodes. Of the 6 cutaneous fascicles from which multiunit afferent activity was recorded, 5 supplied the digits and 1 the palm. In both subjects, spontaneous activity of cutaneous axons developed before the appearance of spontaneous EMG activity. A representative example of the effects of hyperventilation on cutaneous afferent activity, recorded from a fascicle supplying the ring finger, and thenar EMG activity is shown in fig. 1. As indicated by the presence of thenar EMG when the episode of hyperventilation commenced, the thenar muscles were initially not completely relaxed. As the subject hyperventilated and alveolar PCO_2 declined, background neural activity increased, reflecting the spontaneous discharge of cutaneous afferents. At 4.5 min the subject reported paraesthesiae in the face, then in the hands, legs and abdomen. The appearance of spontaneous cutaneous afferent activity and the consequent paraesthesiae occurred

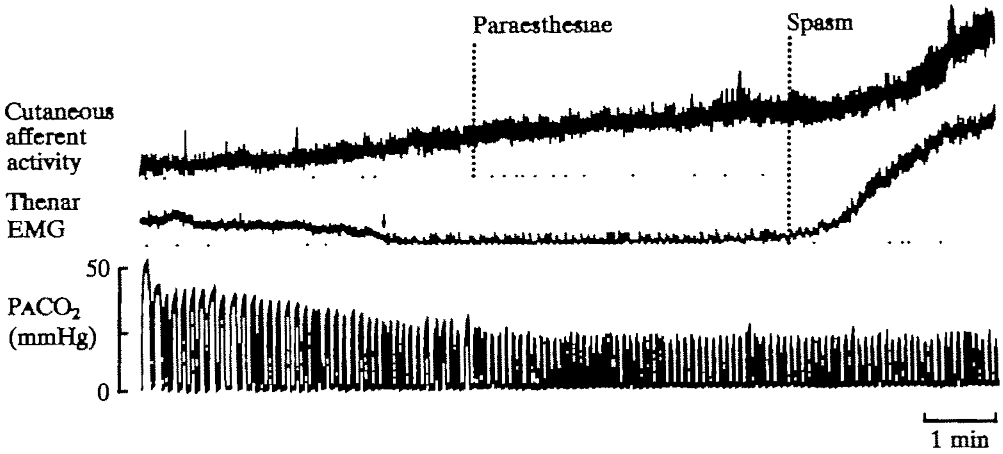

FIG. 1. Integrated neural activity recorded within a fascicle innervating digit IV (*upper trace*), integrated EMG activity recorded with surface electrodes over the thenar muscles (*middle trace*), alveolar PCO_2 (*lower trace*) during voluntary hyperventilation. The arrow in the middle trace indicates voluntary relaxation of the hand, on request. Background neural activity increased progressively as P_ACO_2 declined, reflecting the spontaneous discharge of cutaneous axons. Involuntary EMG leading to a tetanic spasm of the hand developed 8 min after neural activity began to increase. Paraesthesiae were reported first in the face, 4 min before tetany developed in the hand. Integrator time constants were 200 ms.

before the development of spontaneous electromyographic activity of the thenar muscles, associated with the tonic spasm of the hand (Trousseau's sign). At this point a further increase in cutaneous afferent activity occurred, and was associated with the subject reporting severe and extensive paraesthesiae. During the experiment and on replay of the recorded data, the late increase in the integrated neurogram was shown to be not due to contamination of the record by EMG potentials from adjacent muscles. Spontaneous bursts of discharges in cutaneous axons often occurred at the end of a deep exhalation, as indicated in fig. 2. These trains of ectopic discharges were experienced as distinct paraesthetic sensations, referred to the cutaneous field of the nerve fascicle from which mechanically-evoked activity could be recorded optimally.

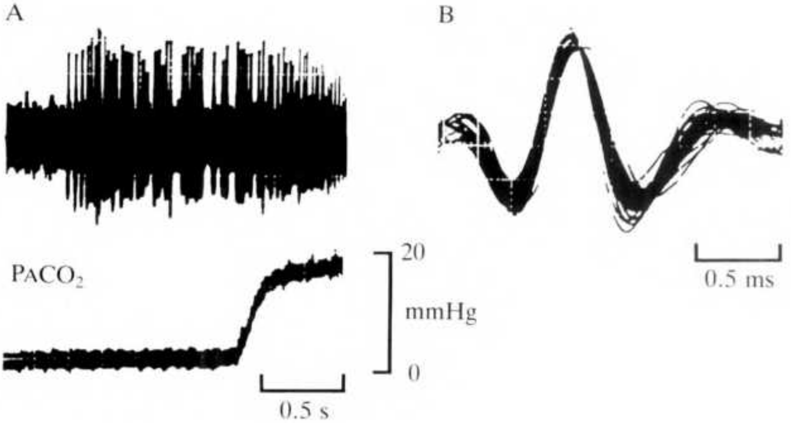

FIG. 2. A, spontaneous bursting discharge of a cutaneous afferent recruited by exhalation in a hypocapnic subject ($P_{A}CO_2$ ~20 mmHg). Fascicle innervating digit IV. The trace commences 1 s after the end of the previous exhalation: the subsequent exhalation commences at the right. B, superimposed action potentials from the discharge train in A. The subject felt the ectopic burst as a discrete paraesthesia, referred to the pulp of the fascicular innervation zone.

Relative changes in excitability of cutaneous and motor axons during hyperventilation

For spontaneous discharges of cutaneous axons and motor units to occur requires an increase in excitability of the axons. In each of 6 subjects, the excitabilities of motor and cutaneous axons, as measured from changes in the compound muscle and sensory action potentials evoked by stimulation of the median nerve at the wrist, increased progressively during voluntary hyperventilation. Increased excitabilities were evident after 1 min, when $P_{A}CO_2$ had declined by only 6–15 mmHg (mean ± SD, 9 ± 3 mmHg). The changes in excitability of cutaneous and motor axons during the first 9 min of hyperventilation are illustrated in fig. 3A, which shows the control potentials and those obtained at 1 min intervals during the developing hypocapnia. In this experiment the control muscle action potential was 1% of maximal, increasing to 11% after 9 min of hyperventilation, and the control sensory action potential was 61% of maximal, increasing to 73%. The mean relative increases in cutaneous and motor axonal excitability

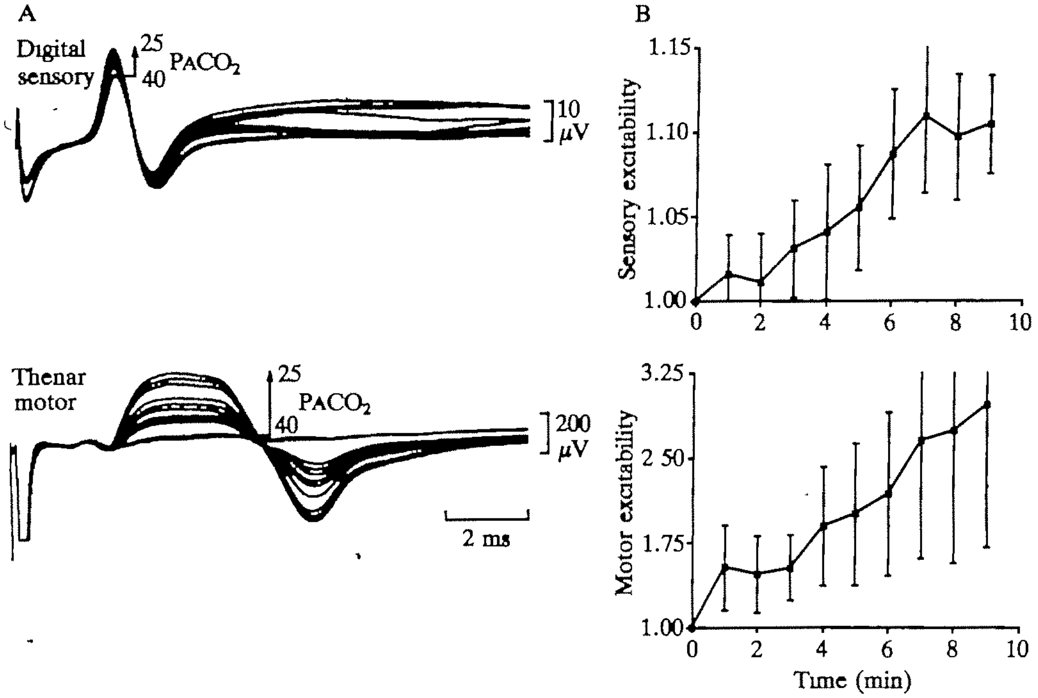

FIG. 3. A, antidromically-conducted compound sensory action potentials recorded over the digital nerves of the index finger (*upper records*), and compound muscle action potentials recorded over the thenar muscles (*lower records*), evoked by constant-current stimulation (25.5 mA) of the median nerve at the wrist in one subject. Each set of traces consists of 3 control records and 9 records obtained at 1 min intervals during hyperventilation The arrows indicate the increases in sensory and motor excitability as alveolar PCO$_2$ declined from 40 to 25 mmHg. B, normalized increases in sensory and motor excitability, as a function of time from the beginning of hyperventilation Excitability was measured as the relative increase in area of the compound action potentials. Data (mean ± SEM) from 6 subjects

with time are represented graphically for all subjects in fig. 3B. Linear regression of excitability versus time was significant for both sets of data ($r \geq 0.966$ for each subject; $P < 0.001$, ANOVA). Overall, the change in motor excitability with time was relatively greater than the change in sensory excitability and in 2 subjects the compound muscle action potential increased in size before any apparent change in the sensory potential. However, this does not establish that motor axons are more sensitive to the excitatory effects of hypocapnia, because changes in motor axon excitability are greatly amplified in an EMG recording; recruitment of only 1 additional axon would be detectable in the compound muscle action potential but not in the sensory potential.

To determine whether the increase in excitability affected low and high-threshold axons equally, stimulus-response curves for the motor and sensory axons were established in the 6 subjects during normal breathing and at a stable level of hyperventilation-induced hypocapnia. The stimulus range extended from motor threshold to a level at which the motor potential was maximal. Data from 1 subject are illustrated in fig. 4. The curve derived from the motor data was displaced to the left during hyperventilation in 5 of the 6 subjects, indicating an increase in axonal excitability across the entire stimulus range. The leftward shift was significant in 4 of the subjects ($P \leq 0.028$), according

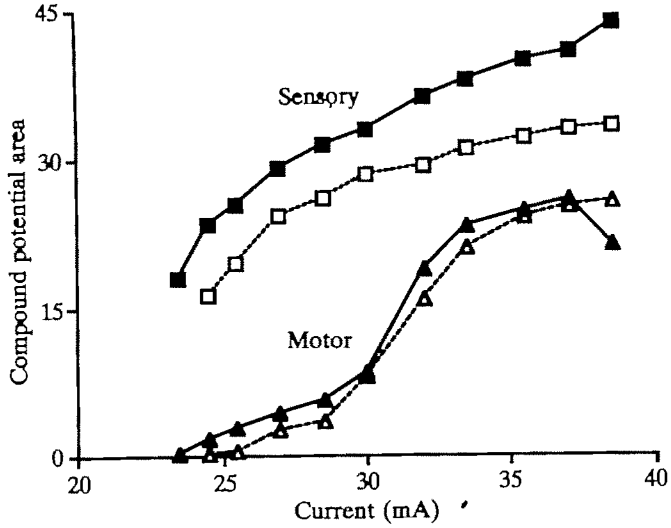

FIG. 4 Stimulus-response curves for sensory and motor axons of the median nerve in 1 subject The stimulus range extended from motor threshold to the level at which the motor potential was maximal. The areas of the sensory and motor compound potentials are expressed in nVs and μVs respectively. Data were obtained during normal breathing (normocapnia) and hyperventilation (steady-state hypocapnia) At each stimulus level, the sensory and motor potentials were larger during hyperventilation. Broken line = normocapnia, continuous line = hypocapnia.

to paired t tests that compared the differences in size of the potentials generated by identical stimulus levels in normocapnic and hypocapnic conditions. The shift in the curve derived from the sensory data was significant for 3 of the 4 subjects in whom a leftward shift was evident ($P \leq 0.003$, paired t test). In all subjects the size of the compound motor and sensory action potentials evoked by the weakest test stimuli was larger during hyperventilation.

Changes in excitability and supernormality of cutaneous axons during hyperventilation

In the second series of experiments, conducted on the same subjects, the orthodromic sensory potential evoked by stimulation of the digital nerves of the index finger was recorded over the median nerve at the wrist. Under normocapnic conditions the recovery cycle of cutaneous axonal excitability was determined for each subject by delivering the test stimulus 1.5–26.0 ms after a maximal conditioning stimulus. The unconditioned test stimulus was set at a level that produced a volley between 25 and 45% (mean ± SD, 35 ± 7%) of the amplitude produced by a maximal stimulus. The pooled data from all 6 subjects is plotted in fig. 5. After the refractory phase there was, in all subjects, an extended period of supernormality, in which the amplitude of the *conditioned* test volley was greater than that of the *unconditioned* volley; no subsequent phase of subnormality was observed with intervals up to 26 ms. The conditioning-test interval producing the maximal degree of supernormality (5–8 ms) was then used to follow changes in supernormality during the hyperventilation. Changes in axonal excitability were determined from changes in the *unconditioned* test volley.

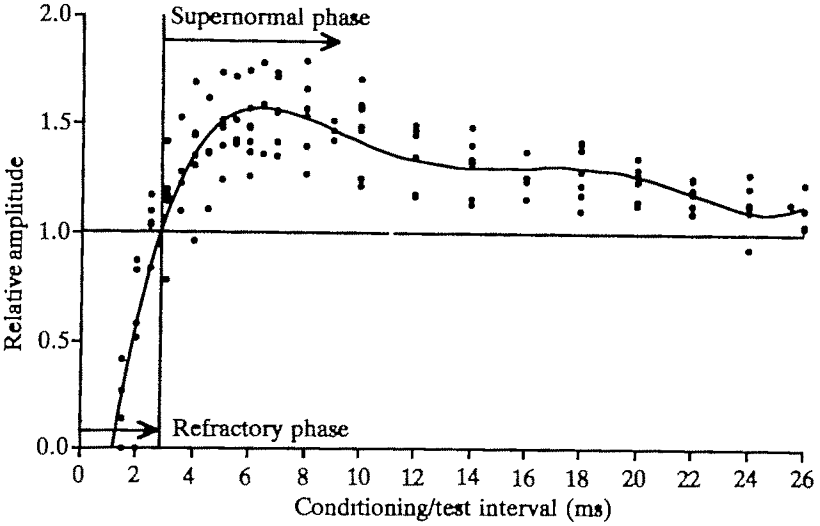

FIG 5. Recovery curve of cutaneous axonal excitability under normocapnic conditions; the curve is a fifth-order polynomial generated from the mean data of 6 subjects. The amplitude of the compound test potential, when preceded by a maximal conditioning stimulus at the interval indicated along the abscissa, is normalized to the size of the unconditioned test potential, evoked by constant-current stimulation of the digital nerves of the index finger and recorded over the median nerve at the wrist After an initial phase of refractoriness, a phase of supernormality began at conditioning-test intervals greater than ~3 ms, during which the amplitude of the conditioned test potential was greater than that of the unconditioned test potential

During hyperventilation the amplitude of both the unconditioned and conditioned test volleys increased (fig. 6A). Axonal excitability increased as $PaCO_2$ declined (fig. 6B), there being a significant inverse linear correlation with $PaCO_2$ in each subject ($r \geq 0.851$; $P \leq 0.007$, ANOVA). As indicated by the higher gradient of the regression line, 1 subject was particularly sensitive to a reduction in $PaCO_2$; in this subject paraesthesiae developed when CO_2 had declined by only 10 mmHg. When measured at a stable level of hypocapnia the stimulus-response curve was displaced to the left in each of 4 subjects in whom a sufficient range of stimulus levels was tested (fig. 7); the displacement was significant in 3 of the subjects ($P \leq 0.007$, paired t test).

Changes in supernormality as a function of $PaCO_2$ are indicated in fig. 6C. The magnitude of the supernormality decreased with $PaCO_2$ and, in 4 subjects, the correlation was significant ($r \geq 0.754$, $P \leq 0.031$, ANOVA). In 2 subjects the correlations were not significant. However, in this paradigm, the degree of supernormality was assessed using a changing test volley, and the degree of potentiation due to supernormality is greater the smaller the test volley (fig. 8A). To eliminate this factor the change in supernormality during hyperventilation was assessed while subjects maintained a stable $PaCO_2$ by determining the change across a range of stimulus levels (using a fixed conditioning-test interval). Fig. 8B presents such data for 2 of the 4 subjects. Supernormality was present when the conditioned test potential was bigger than the unconditioned potential, and its extent at each stimulus level is indicated by the shift from the line of equality (interrupted line in fig. 8B). For none of the 4 were the curves during normal breathing and during hyperventilation significantly different ($P = 0.192-$

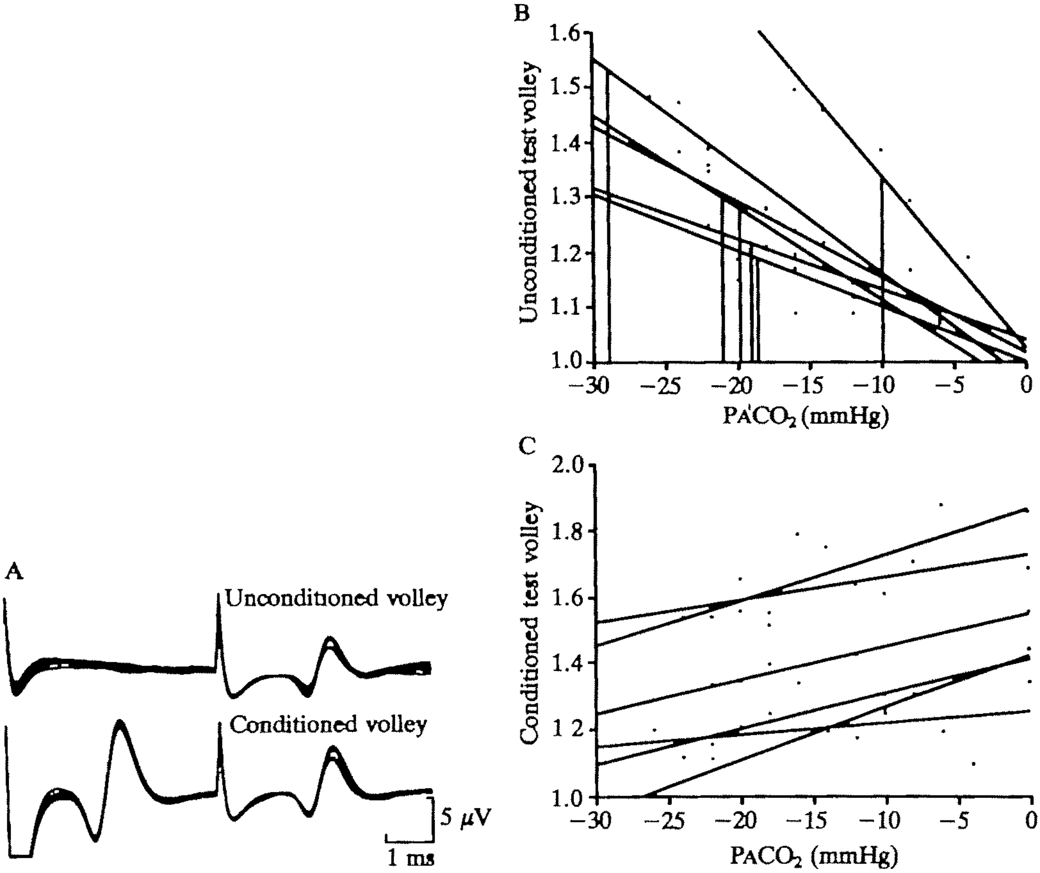

FIG 6. A, hyperventilation-induced increases in the amplitude of the unconditioned test potential (*upper records*) and conditioned test potential (*lower records*) evoked by constant-current stimulation of the digital nerves of the index finger and recorded over the median nerve at the wrist The volley generated by the maximal conditioning stimulus, delivered 5 ms before the test stimulus, was not affected by hyperventilation (*lower records*). Each set of traces consists of duplicate records obtained during normal breathing and 5 records obtained during hyperventilation. B, regression lines relating the excitability of cutaneous axons to the decline in $P_{A}CO_2$ from control Data from 6 subjects. Vertical lines indicate the levels by which $P_{A}CO_2$ had declined when each subject reported paraesthesiae C, regression lines relating the peak supernormality (conditioned/unconditioned test potential) of cutaneous axons to the decline in $P_{A}CO_2$ from control. Data from same 6 subjects in B. In B and C the dots indicate the individual data points for the 6 subjects.

0.749, paired t test; $P = 0.169-0.500$, Mann-Whitney U test), i.e., hyperventilation did not increase peak supernormality. In 1 subject the recovery cycle was plotted for intervals between 1.5 and 26 ms while steady-state hypocapnia was maintained by hyperventilation; hyperventilation did not alter the time course of the cycle.

DISCUSSION

The present study has demonstrated a significant increase in the excitability of cutaneous and motor axons of the median nerve during voluntary hyperventilation in normal subjects.

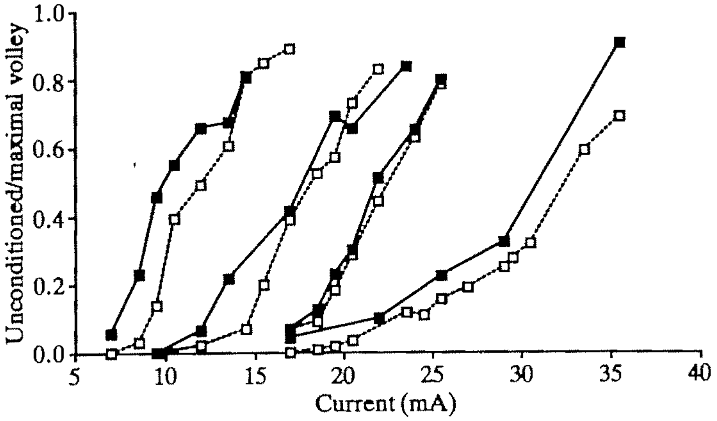

FIG. 7. Stimulus-response curves of cutaneous axonal excitability in 4 subjects. Amplitudes of the unconditioned test volleys are normalized to the amplitude of the volley evoked by a maximal stimulus. Data were obtained during normal breathing (normocapnia) and hyperventilation (steady-state hypocapnia). For each subject the curves were shifted to the left during hyperventilation. Broken lines = normocapnia; continuous lines = hypocapnia.

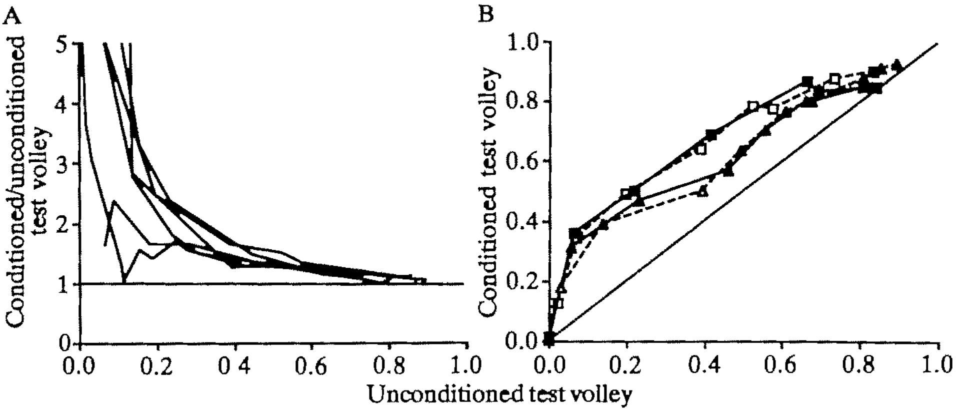

FIG. 8. A, magnitude of peak supernormality as a function of the normalized test volley. With a constant conditioning-test interval, supernormality was greater the smaller the test volley. The ordinate is truncated; some of the curves extend to infinity; B, normalized amplitude of the conditioned test volley versus the normalized amplitude of the unconditioned test volley. Points above the line of identity indicate the magnitude of peak supernormality. Data from 2 subjects, obtained during normal breathing (normocapnia, broken lines) and hyperventilation (steady-state hypocapnia, continuous lines). There was no significant change in the curves during hyperventilation, indicating that there was no change in supernormality other than that attributable to increases in amplitude of the unconditioned test potential.

An increase in excitability of motor axons in human peripheral nerves during hyperventilation has been reported previously (Kugelberg, 1948). The present study extends this observation to sensory axons and demonstrates a significant inverse correlation between axonal excitability and alveolar PCO_2. It is also the first to assess changes in the supernormal phase of the axonal recovery cycle during hyperventilation. An increase in supernormality could precipitate repetitive firing of axons by facilitating the generation of subsequent action potentials (Applegate and Burke, 1989). High-frequency discharges of ectopically active axons have been observed in human peripheral nerves in various conditions associated with paraesthesiae (Ochoa and Torebjörk, 1980; Culp and Ochoa, 1982; Nordin *et al.*, 1984; Burke and Applegate, 1989; present results). However, there was no change in supernormality during steady-state hypocapnia. It can be concluded that the spontaneous discharges of cutaneous and motor axons during hyperventilation depend only on the increases in axonal excitability, and that the supernormal phase determines only the maximal discharge frequencies of the ectopically generated impulses.

Although the findings were consistent across subjects, there was variability in the extent of changes in different subjects, as indicated by the size of the error bars in, for example, fig. 3. This variability could have resulted from a true intersubject difference, but two technical factors undoubtedly contributed. First, subjects were free to choose their own rate and depth of hyperventilation. Secondly, there were inevitably differences in the sizes of the unconditioned test potentials. The extent to which these factors contribute to the overall variability cannot be determined but, whatever its cause, intersubject variability in the extent of the changes is, of itself, of little fundamental importance, given the consistency of the response pattern across subjects.

Mechanism of increase in axonal excitability

Although hyperventilation causes hypocapnia and alkalosis, the favoured interpretation for the increase in axonal excitability relies on the resultant changes in extracellular [Ca^{2+}]. Kugelberg (1948) considered this mechanism on the basis of similarities in the EMG features of tetany induced by hyperventilation and that associated with hypocalcaemia due to hypoparathyroidism; a reduction in serum ionized calcium has been documented during hyperventilation (Fanconi and Rose, 1958; Davis *et al.*, 1970). In addition, paraesthesiae of the face and distal extremities occur during artificially induced hypocalcaemia (Davis *et al.*, 1970; Becker *et al.*, 1974). These latter studies did not note any signs of muscular irritability, presumably because the levels of hypocalcaemia were insufficiently profound. Hyperventilation-induced tetany develops some time after the appearance of ectopic sensory discharges (*see* fig. 1), presumably reflecting a higher threshold for the effects of hypocalcaemia. Differences in the electrophysiological properties of sensory and motor axons have been well-documented, and primarily reflect differences in K^+ conductances (Palti *et al.*, 1980; Neumcke, 1981; Bowe *et al.*, 1985).

The simplest explanation for the effects of Ca^{2+} on axonal excitability is provided by the 'screening' effect of divalent cations on the electric field of the membrane (Ruff, 1986*a*). Frankenhaeuser and Hodgkin (1957) observed that a smaller depolarization was required to open Na^+ and K^+ channels of the squid axon in the presence of a reduced extracellular [Ca^{2+}]. In the amphibian axon, however, low [Ca^{2+}] increases Na^+ but not K^+ conductance (Hille, 1968). In addition to changing the effective surface charge, calcium may directly influence channel kinetics (Ruff, 1986*a*, *b*). For example, if reduced [Ca^{2+}] decreased the fast K^+ conductance (G_{Kf}), then the 'negative accommodation' to a stimulus would increase (*see* Baker and Bostock, 1989); such an increase in axonal excitability could lead to the generation of repetitive discharges in sensory axons (Bowe *et al.*, 1985). Calcium-dependent K^+ channels have been described in the membranes of neuronal cell bodies (Krnjević *et al.*, 1975; Kostyuk, 1984; Ruff, 1986*b*; Latorre *et al.*, 1989), but whether such channels are also present in axonal membranes is not known.

The absence of a change in supernormality during hyperventilation is not surprising, given the proposed mechanisms of action of hypocalcaemia on axonal membranes. In myelinated axons supernormality is associated with a negative (depolarizing) after-potential (Gasser and Erlanger, 1930; Barrett and Barrett, 1982; Bostock and Grafe, 1985; Bowe *et al.*, 1987), attributed to a passive capacitative discharge of internodal membrane, and influenced only indirectly by extracellular Ca^{2+}, K^+ and H^+ (Barrett and Barrett, 1982; Bostock and Grafe, 1985). Admittedly, comparable experimental conditions cannot be reproduced in human subjects (*see*, however, Baker and Bostock, 1989), and there is therefore greater potential for ionic mechanisms to modify supernormality than in organ-bath experiments. Nevertheless, our results are consistent with the finding of Malenka *et al.* (1983) on the unmyelinated parallel fibres of the cerebellum, that a reduction in extracellular $[Ca^{2+}]$ increases their excitability but has no effect on the supernormal period. This does not mean that the supernormal phase of the recovery cycle plays no role in paraesthesiae. It probably still acts to entrain the discharge to the rising slope of the supernormal phase. Ectopically active axons would tend to discharge at frequencies dependent on the time course of their supernormal phase.

Sites of ectopic impulse generation

Possible sites at which an increase in excitability may result in the spontaneous generation of sensory action potentials, and the consequent perception of paraesthesiae, include the generator region of the receptor, the axon and the ganglion cell. Because spontaneous discharges were observed in the peripheral nerve during hyperventilation (fig. 1), and were coincident with the perceived paraesthesiae, any changes in neural excitability at higher levels of the neuraxis cannot be considered causal. Changes in excitability of the peripheral axons certainly occurred, as measured from changes in amplitude of the electrically evoked afferent volley, and were evident *before* the development of paraesthesiae. It is tempting to attribute the ectopic discharges responsible for paraesthesiae to the increase in axonal excitability, much as has been done for tetany by Kugelberg (1948). However, hypocalcaemia increases the excitability of ganglion cells (Bronk *et al.*, 1938) as well as their axons (Katz, 1936. Lehmann, 1937*b*; Brink *et al.*, 1946; Frankenhaeuser, 1957; Frankenhaeuser and Hodgkin, 1957; Hille, 1968). Consequently, it remains possible that the ectopic impulses recorded microneurographically from the cutaneous axons were antidromically conducted action potentials generated at the cell body or, perhaps, orthodromic potentials produced at the receptor generator region. On the other hand, the view that the ectopic discharges are of axonal origin is supported by analogy with Chvostek's sign (increased mechanosensitivity of motor axons in the facial nerve in hypocalcaemia), and with two models of paraesthesiae. Postischaemic paraesthesiae in the hand have been shown to occur independently of the receptor or ganglion cell (Merrington and Nathan, 1949), and the spontaneous paraethesiae following prolonged electrical stimulation of digital nerves are still felt distal to a complete nerve block (Burke and Applegate, 1989).

Hypocalcaemic tetany occurs even when a peripheral nerve is blocked proximally, and is therefore neither of reflex origin nor due to a direct effect on the motoneurons (Kugelberg, 1948). However, effects on the neuromuscular junction or the muscle itself cannot be excluded. A reduction in extracellular $[Ca^{2+}]$ increases the excitability of motor end-plates, rather than the muscle fibres, leading to spontaneous EMG activity (Kuffler, 1944). However, because increases in size of the electrically evoked muscle action potentials in the present study occurred well before the development of spontaneous fasciculation or tetany, it can be concluded that the initial effect of hyperventilation on muscle is via increased excitability of motor axons.

ACKNOWLEDGEMENTS

This work was supported by the National Health and Medical Research Council of Australia.

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(Received November 7, 1989. Revised February 10, 1990. Accepted April 5, 1990)

Brain (1991), 114, 541–556

CORTICOBASAL DEGENERATION

A UNIQUE PATTERN OF REGIONAL CORTICAL OXYGEN HYPOMETABOLISM AND STRIATAL FLUORODOPA UPTAKE DEMONSTRATED BY POSITRON EMISSION TOMOGRAPHY

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SUMMARY

Corticobasal degeneration presents with an asymmetric akinetic-rigid syndrome, apraxia and combinations of supranuclear gaze palsy, myoclonus, and an alien limb. Six patients aged 59–77 yrs, diagnosed on clinical criteria as having corticobasal degeneration, have been studied with positron emission tomography using tracers of dopamine storage capacity and oxygen metabolism. Striatal ^{18}F-6-fluorodopa uptake was reduced in an asymmetric pattern, caudate and putamen being involved in all cases. Uptake into medial frontal cortex was also impaired. Regional cortical oxygen metabolism was most significantly depressed in the superior and posterior temporal, inferior parietal, and occipital associated cortices. Within the frontal lobe, the hypometabolism was chiefly posterior. This unique combination of regional hypometabolism and disruption of the nigrostriatal system is discussed in relation to the clinical features of the disease and is compared with reported findings in other disorders of cognition and movement.

INTRODUCTION

In 1968 Rebeiz *et al.* reported 3 Irish patients who presented in late middle age with variable combinations of an asymmetric akinetic-rigid syndrome, involuntary movements, apraxia, dysarthria and dysphagia, supranuclear palsy and frontal lobe signs. Mental impairment occurred late and the patients came to postmortem after 6–8 yrs. The findings included cortical atrophy in the posterior frontal, paraolfactory and parietal regions. The inferior and medial temporal, hippocampal and occipital regions were normal. Two cases had degeneration of the dentate nuclei, whilst the substantia nigra showed cell loss in all 3. Within the atrophied regions, there was extensive neuronal loss, with abnormal, pale, swollen cells.

Whereas this clinical syndrome shared some features with those described by Steele *et al.* (1964), the disorder of eye movements was less severe, as was the degree of dementia. Whilst the patients described by Steele *et al.* typically presented with axial rigidity and falls, the motor disturbance in these patients began in the limbs and was grossly asymmetric. Furthermore, the pathology in the cases of Rebeiz *et al.* was distinct. In contrast there were clear pathological similarities with Pick's disease, pale, swollen

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cells being found in both. This combination of clinical and postmortem findings was considered novel by Rebeiz *et al.* who described the disease as 'corticodentatonigral degeneration with neuronal achromasia'.

Scully (1985) reported a further patient who presented with rigidity, apraxia, an alien limb, dysarthria and memory impairment. Postmortem findings were of frontal atrophy with swollen achromatic cortical cells. Gibb *et al.* (1989) described 3 further patients with 'corticobasal degeneration' presenting with focal dystonia and myoclonus, an alien hand, and an akinetic-rigid syndrome, respectively. All developed a supranuclear palsy, parkinsonian features and mild cerebellar signs. At postmortem they had frontoparietal atrophy and subcortical cell loss. Abnormal achromatic cells were found in superior frontal, superior temporal and inferior parietal cortices, and subcortical structures including the substantia nigra. A distinctive basophilic inclusion located in the substantia nigra and the locus coeruleus was felt to be unique to this disorder.

Few patients with clinically diagnosed 'corticobasal ganglionic degeneration' have been studied with positron emission tomography (PET). Watts *et al.* (1985) reported 2 patients with 'decreased frontal blood flow and metabolism bilaterally', and Eidelberg *et al.* (1989) described 6 patients with thalamic and inferior parietal hypometabolism contralateral to the most affected limbs. The aim of the present study was to quantitate regional cortical and subcortical oxygen metabolism and striatal ^{18}F-dopa uptake in clinically diagnosed cases of corticobasal degeneration. The hypothesis was that these patients would share common abnormalities on functional images which would distinguish them from previously studied akinetic-rigid and dementing illnesses.

METHODS

Six patients with probable corticobasal degeneration (CBD) were studied with positron emission tomography (PET). Scans were performed on the CTI 931/12/8 (CTI, Knoxville, Tennessee, USA) scanner at the MRC Cyclotron Unit at the Hammersmith Hospital. The performance characteristics of this scanner are as described by Spinks *et al.* (1988). Ethical permission for these studies and for studies on normal volunteers was obtained from the ethical committee of the Royal Postgraduate Medical School, Hammersmith Hospital Approval to administer radiolabelled gases and ligands was obtained from the Administration of Radioactive Substances Advisory Committee of the United Kingdom (ARSAC). Written consent was obtained from all patients and normal volunteers after a full explanation of the procedure.

For all studies, patients were positioned in the scanner with the orbitomeatal line parallel to the detector rings, the head being supported and immobilized in an individually moulded polyurethane support. Each patient underwent 2 scans and we used the same head support for each. Using the bed and gantry coordinates, we tried to position the head in exactly the same position on each occasion. The room was darkened and the position of the patients enabled them only to see the ceiling of the scanner. A 22 gauge arterial cannula was inserted into the radial artery after subcutaneous infiltration with bupivicaine 1%. A 15 min transmission scan was collected using a retractable ^{68}Ga/^{68}Ge ring source. Steady-state emission data were collected for 2 scans each of 10 min duration, during inhalation of $C^{15}O_2$ and $^{15}O_2$ (0.75 $MBq \cdot ml^{-1}$); 10 min washout time followed each scan. A further 6 min scan was collected after a 4 min inhalation of $C^{15}O$. Each scan was reconstructed into 15 planes with a resolution of $8.5 \times 8.5 \times 7.0$ mm without interplane deadspace. Parametric images of regional cerebral blood flow (rCBF), regional cerebral oxygen metabolism (rCMRO$_2$), oxygen extraction ratio (rOER) and regional cerebral blood volume (rCBV) were then calculated from the emission scans using arterial oxygen content and whole blood and plasma counts measured in triplicate during each scan (Frackowiak *et al.*, 1980; Lammertsma and Jones, 1983).

On a separate occasion, each patient underwent an ^{18}F-dopa scan to assess the presynaptic functional integrity of the nigrostriatal system. L-dopa or dopa agonist drugs (when prescribed) were discontinued the night before the scan. Patients received 100 mg of carbidopa 1 h before the study and a further 50 mg immediately before scanning. A 15 min transmission scan was collected and then each patient was given approximately 200 MBq of ^{18}F-dopa by i.v. infusion over 2 min. Dynamic emission scans were collected for the following 124 min divided into 28 time frames. Six healthy volunteers had steady-state oxygen scans and 18 healthy volunteers had ^{18}F-dopa scans under identical conditions.

The rCMRO$_2$ scans were analysed using image analysis software (Analyze version 2 0, BRU, Mayo Foundation, USA) on SUN 3/60 Workstations. A series of cortical and subcortical regions of interest was defined and named from the coplanar stereotaxic brain atlas of Talairach and Tournoux (1988). For each scan the 15 plane image set was interpolated to 43 planes which could then be viewed in transverse, coronal and sagittal planes From this image set the degree and angle of displacement from the intercommisural (ACPC) line was measured (Friston *et al.*, 1989). A series of coordinates were then calculated using Microsoft Excel 1.5 (Microsoft Corp., Redmond, WA, USA) on an Apple Macintosh SE30 computer (Apple Computer, Inc., Cupertino, CA, USA), transferring the atlas coordinates into image space. These regions were transferred to the SUN 3/60 and made available within the Analyze program. Each of the original 15 image planes was then viewed in turn and regions of interest stereotactically placed. The position of each region was verified and adjusted in turn to compensate for minor nonlinear variations in anatomy, such as those resulting from variations in ventricular size. Each region was of size 4×4 pixels (8.2×8.2×7.0 mm) or 5×3 pixels (10.2×6.2×7.0 mm). A total of approximately 400 regions was defined for each brain. For each study data were generated for each named region and returned to the Macintosh SE30 for database calculations of mean values according to cortical region. Regional values were also normalized for intersubject variation in occipital metabolic rate.

The normalized regional values were averaged to produce mean values for frontal, temporal, parietal and occipital (association) cortex. These 4 values for each patient and each control subject were then analysed by analysis of variance (Statview II version 1.02, Abacus Concepts, Inc., Berkeley, CA, USA). Post hoc analysis was performed using unpaired t tests and Mann-Whitney U tests to assess individual regional contributions to the overall patient:control variance (Statview II version 1.02, Abacus Concepts, Inc., USA). The *P* values from t tests were corrected for multiple comparisons using Bonferroni's correction (Godfrey, 1985).

^{18}F-dopa scans were analysed to extract time-activity data in caudate (1 region each side of 4×4 pixels), putamen (3 regions of 4×4 pixels placed contiguously along the axis of each putamen), medial frontal cortex (1 circular region of diameter 8 pixels), occipital lobe (1 circular region each side of diameter 16 pixels) and cerebellum (dimensions as for occipital regions). The caudate and putamen region of interest sizes were determined from the size of regions which match the striatal dopa uptake in healthy subjects. They were positioned by eye on the basis of the tracer activity as seen, with reference to the neuroanatomical atlas of Talairach and Tournoux (1988). In all cases, striatal activity was visible on 3 adjacent planes. The activity in the lowest 'ventral striatum' plane was not used in this analysis. The regions of interest were defined on the upper 2 adjacent planes and average values for each anatomical structure were calculated. Regional time activity curves were plotted and the data from 30 to 90 min were analysed using a multiple time graphical analysis approach developed by Patlak and Blasberg (1985). Occipital tissue activity was used as a nonspecific reference input function (Brooks *et al* , 1990).

All patients underwent detailed neurophysiological assessment (which is to be reported elsewhere).

CASE REPORTS

The diagnosis of CBD was made in these patients on the basis of an asymmetric akinetic-rigid syndrome with apraxia and combinations of a supranuclear gaze palsy, myoclonus, or an alien limb.

Case 1

A 56-yr-old woman complained of clumsiness of the right hand. Over the following 4 yrs she developed a severe and predominantly right-sided akinetic-rigid syndrome, with limb apraxia, myoclonus and an apraxic dysarthria. She subsequently developed expressive dysphasia. Formal psychological testing at the age of 58 revealed verbal IQ 78 and performance IQ 96, poor visual memory (scoring 35/50 using a forced choice recognition test for faces) and impaired verbal fluency (naming items beginning with a specific letter). Subsequent formal psychological testing became impossible due to anarthria, though she seemed to understand what was said and was able to, for example, choose between colours indicating correctly with eye movement. She developed a supranuclear gaze palsy, with difficulty initiating both horizontal and vertical saccades, and head movement synkinesis. She had a florid visual rooting reflex. CT scan showed generalized cerebral atrophy and a small area of low attenuation in the posteromedial left temporal lobe. PET scanning was performed at the age of 60. Immediately following this she underwent frontal craniotomy and cortical biopsy. The biopsy included cortex and subcortical white matter Cortical perivascular spaces were dilated but the normal layered arrangement of nerve cells and myelinated fibres was preserved. In both grey and white matter (particularly the latter) there was an increase in astrocyte numbers. Abnormal cortical cells of two types were seen. Some were swollen and achromatic with peripheral nuclei and almost complete loss of Nissl substance; these were mostly situated in the deeper cortical layers. Others were moderately swollen and contained an accumulation of cytoplasmic material displacing the nucleus. Within some of the moderately swollen cells this material stained with RT97, while the swollen cells either did not take up stain, or showed only a faint positive staining. Occasional cells contained neurofibrillary tangles but no senile plaques were seen. Electron microscopic examination of cortex identified a small number of pale cells, which did not contain Pick bodies. Although caution must be exercized before extrapolating from this single cortical biopsy to the remainder of this patient's brain, these pathological findings are in keeping with the diagnosis of CBD.

Over the year following her biopsy and PET studies she deteriorated further. She had no arm movement, but alien movements of the left leg. Bladder and bowel control were preserved; she still watched television and showed amusement in context.

Case 2

A 52-yr-old man complained of stiffness, slowness and shaking of the left hand. Over 6 yrs the left arm became functionally useless, adopting dystonic postures and exhibiting spontaneous and stimulus sensitive myoclonus. The left arm became akinetic, rigid and mildly dyspractic as did both legs. After 3 yrs he developed dysarthria and vocal apraxia. He had facial apraxia, eyelid apraxia (at times being completely unable to close his eyes) and impaired voluntary eye movements (with saccadic intrusion into pursuit movements and slow saccades in all directions). He demonstrated forced rooting and pouting to visual stimuli, forced grasping and brisk palmomental reflexes Formal psychometry when aged 59 revealed a verbal IQ of 95. He completed 2 nonverbal tests (picture completion and picture arrangement) on which he obtained an average and bright average score, respectively, but a complete assessment of performance IQ could not be made because of his myoclonus and apraxia. He performed slowly on tests involving nonverbal reasoning (advanced progressive matrices). A CT scan showed generalized cerebral atrophy.

Case 3

This patient was Sri Lankan in origin, but fluent in English. At the age of 53 yrs she complained of pain and difficulty using the right arm. It became rigid, with action and stimulus sensitive myoclonus, pain and reduced peripheral sensation. She developed mild axial and limb rigidity, with retropulsion on walking. Speech became slow and slurred. On formal psychometry testing at age 57 her verbal IQ was 100 and performance IQ 84. Visual recognition memory was impaired (scoring 39/50 on forced choice recognition test for faces) and she performed poorly on tests sensitive to frontal lobe dysfunction (achieving 2 categories on the Weigl sorting test but with inability to name the first rule; concrete explanation of proverbs; only able to name 7 words beginning with 'S' in 60 s). Whilst eye movements were normal at the time of her scans, over the following year she developed impaired up and downgaze to command and impaired upward pursuit movement. She had head deviation in the direction of slow movement on caloric testing, suggesting disinhibition of vestibulospinal responses. Her abilities on formal psychometric testing deteriorated further and verbal IQ fell to 92 with no change in performance IQ. A CT scan showed generalized cerebral atrophy and mild calcification of the basal ganglia.

Case 4

A 68-yr-old man developed progressive weakness and stiffness of the right leg, which by the age of 72 showed dystonic spasms and stimulus sensitive myoclonus. It was also spastic with clonus. Verbal IQ was 142 and performance IQ 136. The right leg became apraxic with involuntary spasms and alien behaviour. Axial rigidity with rigidity and apraxia of the right arm followed. Speech was unaffected. He developed frequency and urgency of micturition and underwent prostatectomy with some improvement. Repeated CT scans were normal. He was scanned at age 73. Subsequently he became chairbound due to apractic rigidity of both legs and developed marked apraxia of both arms, as well as an anomic aphasia. He had forced groping and a severe grasp reflex. He developed a supranuclear palsy for both horizontal and vertical saccades, with saccadic intrusion into pursuit movements in all directions.

Case 5

A 73-yr-old man complained that over the last year his left hand had slowly become useless. His left hand would involuntarily 'wander', grasping hold of objects and sometimes interfering with the actions of his right hand. He had an akinetic-rigid syndrome affecting all limbs. He had saccadic intrusion into horizontal pursuit movements and a supranuclear downgaze palsy. Formal psychometric tests revealed verbal IQ 96 and performance IQ 103. He had constructional apraxia (apraxic writing, drawing and block design subtest of WAIS), weak verbal memory (able to name 2/12 famous faces despite recognizing them) and impaired performance on tests thought to be sensitive to frontal lobe dysfunction (poor word fluency, interpretation of proverbs and responses on cognitive estimates). He had frontal release signs, including utilization behaviour; he would take a pair of glasses held before him, exchange them with his own and repeat this action until the second pair of glasses was taken out of his sight. A CT scan showed mild generalized cerebral atrophy and basal ganglia calcification.

Case 6

A 77-yr-old woman developed difficulty walking and a 'useless' right hand. She had an akinetic-rigid syndrome affecting the arms more than the legs, with bilateral arm apraxia and postural instability. When playing darts she found that she could not let go of the darts with her right hand. Alien movements developed in the right arm which would 'wave around' on its own and grasp hold of nearby furniture. She became dysarthric due to orolingual apraxia. Eye movements were impaired with poor up and downgaze, saccadic intrusion into smooth pursuit and difficulty initiating saccades to command. Formal psychometry when aged 79 measured her verbal IQ 87. She was severely dyspraxic. With her left hand she was unable to pick up or hold objects appropriately, imitate hand gestures or execute simple acts to command. This precluded formal assessment of performance IQ. She performed poorly on early visual information processing tasks (recognizing 1/5 fragmented letters and being unable to judge the distance of objects relative to others) and on tasks thought to be sensitive to frontal lobe dysfunction (word fluency and interpretation of proverbs). A CT scan showed generalized cerebral atrophy and patchy low density changes in the white matter of both hemispheres.

RESULTS

The principal clinical signs of the 6 corticobasal patients are summarized in Table 1. Neurological assessment of the control subjects was normal in all cases. The control subjects did not undergo formal psychometric testing, but scored 30/30 on the Mini-Mental Test (Folstein *et al*., 1975).

Fig. 1 shows images of $CMRO_2$ for Case 3, illustrating the typical pattern of

TABLE 1. CLINICAL SIGNS*

Case	*1*	*2*	*3*	*4*	*5*	*6*
Age (yrs)	59	59	57	73	74	79
Akinetic-rigid	+	+	+	+	+	+
Supranuclear gaze palsy	+	+	(+)	(+)	+	+
Alien limb	−	−	−	+	+	+
Dyspraxic limb	+	+	+	+	+	+
Myoclonus	+	+	+	−	−	−
Dysphasia	+	−	−	(+)	−	−
Dementia	−	−	−	−	−	−
Frontal lobe signs	+	+	−	+	+	−

* This table indicates the distribution of clinical signs in the patients studied. (+) = signs which have developed subsequent to PET studies.

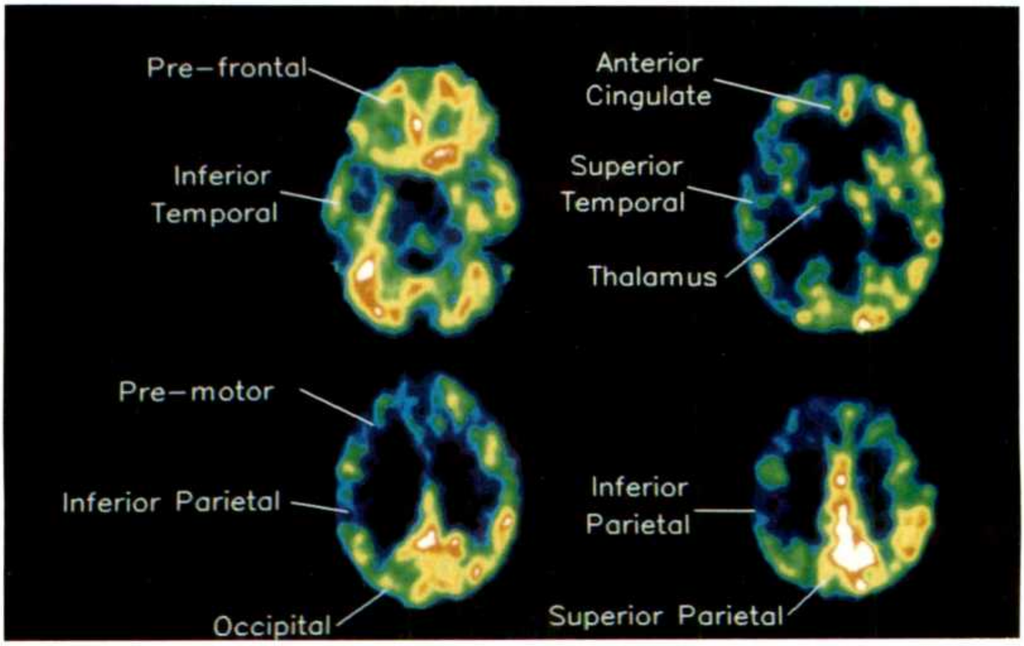

FIG. 1. $CMRO_2$ in *Case 3* showing marked asymmetry with focal hypometabolism affecting premotor cortex, anterior cingulate, superior temporal and inferior parietal cortex and left thalamus. The superior parietal cortex and occipital cortex are well preserved.

hypometabolism with relative sparing of the occipital, inferior temporal and superior parietal cortices. The changes seen are asymmetric, a consistent finding in this patient group (data not shown). The level of global $CMRO_2$ in our patients varied from 2.1 to 3.3 ml oxygen $\cdot$ 100 $ml^{-1} \cdot min^{-1}$. The mean global $CMRO_2$ of 6 healthy volunteers was 3.1 ± 0.2 ml $\cdot$ 100 $ml^{-1} \cdot min^{-1}$.

In view of the wide variation in global $CMRO_2$, regional cortical data were further analysed after normalization to mean (left and right) primary visual cortex (area 17) for both healthy volunteers and patients. Typically 6 or 7 regions of 16 pixels each were drawn on this area for each hemisphere. The primary visual cortex was chosen for this normalization procedure, because primary visual function was normal in all of our patients, and because this region had metabolic values which were normal in 4 patients and only significantly low on raw $CMRO_2$ values in one hemisphere each of the 2 patients with the lowest global values (Cases 3, 6). We did not use global $CMRO_2$ for normalization as such a procedure would render the 'normal' side hypermetabolic in patients with severe and highly asymmetric disease.

An analysis of variance comparing diagnosis (normal vs CBD) with mean normalized frontal, temporal, parietal and occipital cortical $CMRO_2$ values as repeated measures gave an overall significance of $P = 0.0114$.

Table 2 gives a summary of the results of region-of-interest analysis for normalized mean $CMRO_2$ for 6 healthy volunteers and the CBD patients. Data are presented according to functionally related areas and whole lobe values. The t test P values for

TABLE 2 NORMALIZED REGIONAL CMRO$_2$ VALUES[1]

		Corticobasal degeneration	
	Normal values	*Mean ± SD*	*P value*
Primary sensory and motor cortex			
Visual (17)	100 ± 00	100 ± 00	0.1650
Auditory (41, 42)	110 ± 20	82 ± 06	0.0100
Sensorimotor (1–4)	85 ± 03	71 ± 10	0.0083
All	91 ± 03	81 ± 06	0.0120
Visual association cortex			
Occipital (18)	101 ± 01	90 ± 05	0.0013
Occipital (19)	98 ± 01	86 ± 05	0.0006*
Posterior temporal (37)	107 ± 05	85 ± 09	0.0008*
Middle temporal (21)	100 ± 05	84 ± 06	0.0013
Inferior temporal (20)	92 ± 07	84 ± 08	0.1054
All	100 ± 01	87 ± 05	0.0002*
Auditory association cortex			
Superior temporal (22)	103 ± 02	81 ± 08	0.0001*
Somatosensory association cortex			
Superior parietal (5, 7)	96 ± 05	87 ± 10	0.0913
'Higher' association cortex			
Inferior parietal (39)	101 ± 03	84 ± 05	0.0001*
Inferior parietal (40)	94 ± 03	76 ± 09	0.0011*
All	97 ± 03	81 ± 07	0.0006*
Allocortex			
Temporal pole (38)	86 ± 08	67 ± 05	0.0013
Hippocampus (34)	79 ± 11	68 ± 08	0.1060
All	83 ± 05	68 ± 05	0.0007*
Premotor cortex			
Supplementary motor (6 med.)	83 ± 08	67 ± 11	0.0204
Premotor (6 lat.)	87 ± 05	73 ± 11	0.0208
Broca's (44)	92 ± 05	75 ± 13	0.0112
Eye fields (8)	84 ± 04	73 ± 10	0.0504
All	86 ± 04	72 ± 10	0.0133
Prefrontal cortex			
Superior frontal (9)	92 ± 05	76 ± 10	0.0090
Midfrontal (46)	93 ± 03	81 ± 11	0.0375
Inferior frontal (45)	92 ± 04	79 ± 13	0.0526
Frontal pole (10)	88 ± 03	79 ± 11	0.0893
Orbital frontal (11)	96 ± 08	79 ± 14	0.0343
Orbital frontal (47)	91 ± 08	86 ± 13	0.4281
Medial frontal (32)	93 ± 06	75 ± 07	0.0018
All	91 ± 01	79 ± 09	0.0101
Anterior cingulate (24)	82 ± 07	66 ± 07	0.0025
Inferomedial parietal (31)	111 ± 05	87 ± 04	0.0001*
Caudate	95 ± 08	93 ± 08	0.6530
Putamen	108 ± 03	105 ± 12	0.5242
Thalamus	96 ± 07	82 ± 12	0.0302
Cerebellum	107 ± 20	104 ± 08	0.7159
Corona radiata	44 ± 02	35 ± 05	0.0048
Frontal cortex	87 ± 04	76 ± 09	0.0201
Temporal cortex	104 ± 03	85 ± 08	0.0004*
Parietal cortex	99 ± 02	83 ± 04	0.0001*
Occipital cortex	99 ± 00	90 ± 04	0.0004*
Global	98 ± 01	83 ± 06	0.0002*

[1] CMRO$_2$ as % of rate in primary visual cortex for 6 patients and 6 healthy controls (controls aged 63 ± 6 yrs) *P* values are given for two-tailed t test. Similar values were obtained using Mann-Whitney U test. * Indicates *P* values significant at the $P < 0.05$ level after applying Bonferroni's method to correct for all 43 comparisons.

each region are also given and those regions for which the P values are significant after applying Bonferroni's correction are indicated. The temporal, parietal and occipital (association) cortices are all significantly hypometabolic by this analysis. Within the temporal lobe, the principal abnormality is within posterior and superior gyri. The parietal abnormality is inferior. Taking the mean of the 2 hemispheres, none of the frontal lobe regions studied are significantly hypometabolic after applying Bonferroni's correction, though there are several regions which approach this level of significance.

The uptake of ^{18}F-dopa into caudate, putamen and medial frontal cortex is given in Table 3. The laterality of the most severe symptoms and signs is shown for each case. In Cases 1–4 and 6, caudate ^{18}F-dopa uptake was most severely impaired contralateral to the clinically most severely affected side. In Cases 2–6, putamen ^{18}F-dopa uptake was also most severely impaired contralateral to the principal clinical signs, while in Case 1, putamen ^{18}F-dopa uptake was symmetrically impaired. An overall analysis of variance comparing patients vs controls for left and right putamen and medial frontal

TABLE 3. ^{18}F-DOPA UPTAKE VALUES*

	Mean ± SD normal	*Case 1 (right)*	*Case 2 (left)*	*Case 3 (right)*	*Case 4 (right)*	*Case 5 (left)*	*Case 6 (right)*	*Mean (n = 6)*	*P*
Caudate left	0.0119 ± 0.0017	0.0041	0.0098	0.0045	0.0056	0.0080	0.0092	0.0069	0.007
Caudate right	0.0115 ± 0.0023	0.0068	0.0076	0.0083	0.0082	0.0110	0.0092	0.0085	0.029
Putamen left	0.0103 ± 0.0011	0.0047	0.0069	0.0048	0.0036	0.0088	0.0074	0.0060	0.007
Putamen right	0.0103 ± 0.0011	0.0047	0.0048	0.0087	0.0072	0.0068	0.0082	0.0067	0.007
Caudate mean	0.0117 ± 0.0014	0.0054	0.0086	0.0064	0.0069	0.0095	0.0092	0.0077	0.007
Putamen mean	0.0103 ± 0.0011	0.0047	0.0058	0.0067	0.0054	0.0078	0.0078	0.0064	0.007
Medial frontal cortex	0.0027 ± 0.0008	0.0014	0.0016	0.0009	0.0016	0.0022	0.0024	0.0017	0.007

* Values shown are influx constants (Ki) for ^{18}F-dopa uptake into the regions listed, using multiple-time graphical analysis method of Patlak and Blasberg (1985) with occipital cortex as reference to input function. Data for the 6 patients are compared with data from 18 controls (9 male, 9 female, mean age 65 ± 7 yrs). For each patient, the laterality of the first and most severely affected limb is given in parentheses; t test P values given are Bonferroni-corrected for multiple comparisons.

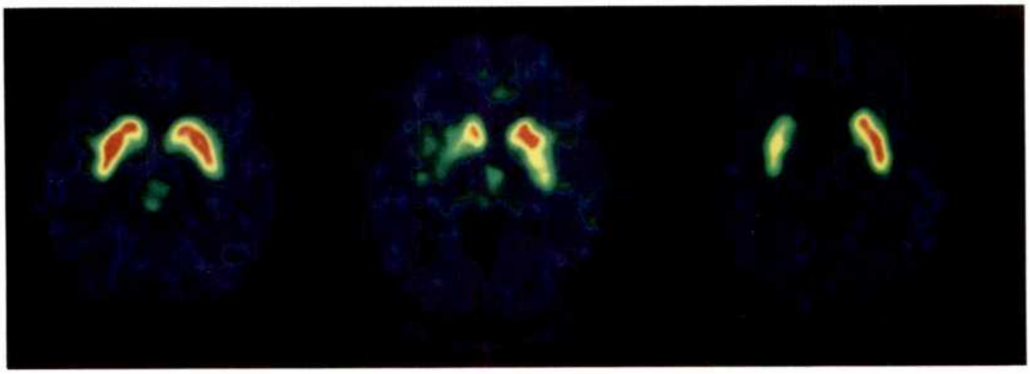

FIG. 2. ^{18}F-Fluorodopa uptake into caudate and putamen in a normal subject (*left*), a patient with typical Lewy body PD (*middle*) and a patient with CBD (*right, Case 1*). The patient with PD has a fairly symmetric loss of putamen uptake with relative preservation of caudate uptake while the patient with CBD shows severe asymmetry, and reduced dopa uptake into both caudate and putamen.

cortex gave an overall significance level of 0.0001. Post hoc region-by-region t tests corrected for multiple comparisons gave P values as given. All are significant at at least the $P < 0.05$ level. Fig. 2 illustrates the uptake of dopa into striatum in a normal subject, a patient with typical Lewy body Parkinson's disease (PD) and a patient with CBD (Case 1). Whereas the patient with PD has fairly symmetric loss of putaminal uptake with relative preservation of caudate uptake, the patient with CBD shows severe asymmetry, with little dopa uptake into caudate or putamen contralateral to the clinically most affected side.

DISCUSSION

Clinical features

The clinical features of our cases are comparable with those of Rebeiz *et al.* (1968), Scully (1985) and Gibb *et al.* (1989), and are distinct from other movement disorders. Our patients were markedly asymmetric in their disabilities, sometimes with one limb progressing to complete functional incapacity before the development of contralateral symptoms. By the time of their PET studies all patients had developed signs of bilateral akinetic-rigid syndrome and all had apraxia of one or more limbs. Three had developed an alien arm and 1 an alien leg. Three had myoclonus and 4 had a supranuclear gaze palsy. Communication skills were impaired in 4 due to dyspraxic anarthria and a further patient became dysphasic. Four developed memory impairment. Three had frontal release signs, 1 showing utilization behaviour, but none were incontinent and personality was preserved in all cases. Formal psychometric assessment revealed a variety of deficits on tasks thought to reflect frontal lobe or other cerebral dysfunction, but in no case was the patient clearly demented.

CT scans showed generalized cerebral atrophy in Cases 1, 2, 3, 5 and 6, with focal changes in Cases 1 and 6. In all cases both the anatomical extent and the severity of the changes seen on CT were exceeded by the changes of PET. Although cerebral atrophy will have an effect on the measurement of regional metabolic rate due to partial volume effects, we have attempted to minimize this by using groups of small regions of interest, rather than larger 'standard templates' based on normal scan data. Cases 3 and 5 showed slight calcification in the basal ganglia, similar to that described as an incidental finding in individuals of this age by Brannan *et al.* (1980).

PET findings and clinical:PET correlates

The most consistent PET findings were a reduction in ^{18}F-dopa uptake into both caudate and putamen and a reduction of CMRO_2 in particular cortical regions. Most severely affected were the superior temporal lobe and inferior parietal lobe. Intersubject variations were of course noted, and these are discussed below in relationship to the associated clinical features.

Akinetic-rigid syndrome. The accumulation of ^{18}F-dopa into striatum reflects its uptake into dopaminergic neurons, conversions into ^{18}F-dopamine by dopa decarboxylase, and subsequent concentration and storage in terminal neurotransmitter vesicles (Firnau *et al.*, 1987). Reduced uptake in the caudate and putamen are believed to represent a reduction in the number of functioning nigrostriatal dopaminergic neurons (Leenders *et al.*, 1986). In PD there is a mean reduction to 40% of normal uptake into the putamen, whilst uptake into the caudate is relatively preserved (Leenders *et al.*, 1989). In our corticobasal patients we found almost as great a reduction in caudate as in putamen ^{18}F-dopa uptake. Rebeiz *et al.* (1968) reported only trivial pathological findings in the caudate, putamen and globus pallidum of their patients (a few scattered foci of cavitation in Case 1 and a few disintegrating cells in the globus pallidus of Case 2) but marked cellular loss in the lateral two-thirds of the substantia nigra. Scully (1985) also found considerable loss of nigral neurons with only slight gliosis in the basal ganglia. Gibb *et al.* (1989) described mild to moderate diffuse loss of pigmented cells in the substantia nigra, but also neuronal loss with gliosis in the striatum (especially the putamen) and the globus pallidus. These findings are thus all consistent with a presynaptic nigrostriatal deficit in this condition, with variable involvement of the striatum and globus pallidus.

Patients with early PD typically show asymmetry in their striatal ^{18}F-dopa uptake. However, the degree of asymmetry seen in these patients would be atypical with advanced PD, though consistent with the degree of clinical asymmetry and indeed the asymmetry of cortical hypometabolism seen with PET (*see* fig. 1).

If the defect of the striatal dopaminergic system were purely presynaptic, it might be expected that these patients would respond well to L-dopa therapy. This was not the case, however, and although our patients were given a trial of L-dopa in no case was there a major or sustained improvement. An explanation for this therapeutic failure could be a concomitant loss of postsynaptic receptors (though the limited pathological evidence discussed above suggests that there is variable involvement of the intrinsic cells of the striatum). An alternative explanation is that the coexistent cortical deficits, such as those of premotor and medial frontal cortex, prevent the return to normal function with simple dopaminergic replacement.

Dyspraxia. Whilst the classic description of apraxia demands that the limb should have no other motor or sensory signs, we feel confident in describing this finding in all of our patients despite their coexisting akinetic-rigid syndrome. In all cases, either at the time of PET scanning or earlier in their clinical course, there was clear evidence of gross disruption of voluntary movement beyond that which might be explained by concomitant motor or sensory signs. Indeed, such severe apraxia of a rigid limb, which to our knowledge has not been described in PD, multiple system atrophy or Steele-Richardson-Olszewski disease, is one of the clues to the diagnosis. In some cases the rigidity subsequently increased to a degree that a confident definition of apraxia could no longer be made. Previous descriptions of this condition have included apraxia, such as in Case 1 of Rebeiz *et al.* (1968) who was apraxic when trying to walk and on 'other volitional acts' and in some of the cases reported by Watts *et al.* (1985).

Apraxia has been described in patients with focal lesions in a number of cortical sites which were hypometabolic in our patients. Thus Freund and Hummelsheim (1985) described patients with 'limb-kinetic apraxia in patients with lesions in premotor cortex, who had temporal deordering of proximal and distal arm muscles with bibrachial, but not bimanual incoordination'. Agostoni *et al.* (1983) described 7 patients with ideomotor apraxia (5 also had constructional apraxia and one buccofacial apraxia) who on CT evidence had ischaemic lesions or intracerebral haemorrhages limited to basal ganglia and/or thalamus. Parietal lesions are perhaps more commonly cited as leading to ideomotor apraxia, though a further category of movement disorders in patients with parietal lesions may occur which does not depend on the side of the lesion, and may comprise ataxia, postural instability and muscle hypotonia (*see* Freund, 1987). All our patients had major parietal hypometabolism and it is possible that this explains much of their motor disturbance; but since in all cases there were additional deficits within the frontal premotor and motor areas and affecting the nigrostriatal pathways, it is difficult to draw precise correlations between particular patients' motor failings and the anatomical site of their pathology.

Alien limb. The patient described by Scully (1985) was said to have apraxia of the left hand, although the description suggests alien behaviour of this limb; when asked to imitate the examiner's hand posture, after a delay of 5 – 10 s he lifted his hand and scratched his scalp. He registered disgust, saying 'I didn't mean to do that, it seems to have a mind of its own'. The alien limb (la main étrangère) was described by Brion and Jedynak (1972) in 3 patients with tumours of the corpus callosum. Each had 'uncooperative' behaviour of the left hand. Similar phenomena involving the left hand have been described by Watson and Heilman (1983) in the few weeks following commisurotomy for severe epilepsy. In contrast, the right hand displayed alien behaviour in 2 patients reported by Goldberg *et al.* (1981) following infarction of the medial part of the left frontal cortex and in 3 patients reported by McNabb *et al.* (1988) after dominant anterior cerebral artery territory infarction. The term 'medial frontal cortex' was used to include the supplementary motor area (6, medial) and the anterior cingulate (area 24) in addition to the medial frontal cortex (area 32). It may be that the transient alien phenomena after commisurotomy are related to retraction of the nondominant right hemisphere intraoperatively in the course of exposing the corpus callosum for section (Wilson *et al.*, 1977). All 5 of the patients of Goldberg *et al.* and those of McNabb had a transcortical motor aphasia, which was not seen in any of our patients. Likewise the vascular patients all presented acutely, whereas in each of our patients the alien phenomena slowly worsened over a period of months. In our patients with alien limbs, we found hypometabolism in the medial frontal cortex (area 24) bilaterally in Cases 1 and 4, and contralateral to the affected limb in Case 5. The contralateral supplementary motor area was focally hypometabolic in Case 1. These findings are in agreement with the sites of vascular damage reported both by Goldberg *et al.* and McNabb *et al.* They are also consistent with the suggestion that the medial frontal cortex and supplementary motor area are involved in the execution of learned motor programs and that damage to these areas could release learned motor programs from voluntary control.

Supranuclear gaze palsy. Four of our patients had a supranuclear gaze palsy at the time of their PET scans. Three patients had saccadic intrusion into pursuit movements (Cases 2, 5, 6) and 3 had slow saccades in all directions (Cases 1, 2, 6). One patient (Case 1) had eye-head synkinesis, which was also noted in Case 1 of Rebeiz *et al.* (1968). Of the 2 patients who had normal eye movements at the time of PET, both have subsequently developed a supranuclear gaze palsy. A supranuclear gaze palsy was recorded in 2 of the patients described by Rebeiz *et al.* (1968) and in all the patients described by Gibb *et al.* (1989). The complexity of the corticobulbar pathways for gaze hinders any functional correlation of the sites of pathology and the gaze palsies seen, particularly as the caudal part of the pathways cannot presently be imaged with this technique. Four of our patients, however, did have significant hypometabolism within area 8 (bilaterally in Cases 1 and 2), which includes the primary frontal eye fields. Others had more diffuse frontal and parieto-occipital lesions.

Aphasia. Communication was impaired at the time of scanning in 4 of our patients. Case 1 had an expressive dysphasia, in addition to a severe dysarthria. In Cases 1, 2 and 6 there was an articulatory apraxia. Following scanning, Case 3 has developed severely impaired and monosyllabic speech and Case 4 has developed an anomic aphasia. It is of interest that Broca's area (area 44) was abnormal on the left side in Cases 1, 2 and 3, the most severe hypometabolism being in Case 1 (data not shown).

Myoclonus. Myoclonus was described in 1 of the cases reported by Gibb *et al.* (1989), and was evident in this report in Cases 1, 2 (spontaneous and stimulus sensitive) and 3 (action and stimulus sensitive). Electrophysiological studies, to be reported elsewhere, suggested a spinal interneuronal aetiology for the myoclonus in Case 2, and possibly a subcortical origin in Cases 1 and 3. It is of interest that the thalamus was hypometabolic on the left side in both Cases 1 and 3, although it was normal in Case 2. CMRO$_2$ was normal in all subcortical structures in all other patients, except for the left putamen in Case 1, the right putamen in Case 2 and the left caudate in Case 7.

Differential diagnosis

Table 4 summarizes the PET findings in related conditions with a disturbance of cognition and/or movement. Whilst the clinical presentation of patients with CBD is quite unlike that of Pick's disease, the histological similarity between these diagnoses raises the possibility that they represent overlapping conditions. Kamo *et al.* (1987) studied a single patient with advanced Pick's disease using PET and ^{18}F-2-fluoro-2-deoxyglucose. When LCMR-glu values for this patient were normalized to cerebellar values, frontal lobe activity was 49% of normal while temporal, parietal and occipital lobe values were normal. Clearly our patients with CBD show less severe frontal changes, and greater parietal lobe hypometabolism. The cortical pattern of hypometabolism in CBD shares some features with dementia of Alzheimer type. Thus Haxby *et al.* (1988) reported parietal and lateral temporal hypometabolism in patients with early disease and premotor and parietal hypometabolism in moderate disease, extending to involve all regions except the occipital lobe in advanced disease. However, the striking predilection of CBD to involve the *inferior* parietal lobe has not, to our knowledge, been reported in Alzheimer's disease.

PET studies in patients with PSP have consistently reported frontal and sometimes global, including parietal, hypometabolism. Parietal hypometabolism has not, however, been reported as the dominant cortical finding in PSP. Blin *et al.* (1988) reported PET findings in 41 patients with clinically determined PSP. They reported a 14% reduction in cortical metabolic rate for parietal associative cortex and an 18% reduction for temporoparietal cortex. Frontal regions showed a 24% reduction. When the regional metabolic ratios were normalized to occipital cortex, only the frontal regions were significantly abnormal. In our CBD patients the reverse is true. When normalized to the occipital cortex, the frontal lobe is the only lobe *not* significantly hypometabolic. Unlike the contrasting pattern of metabolic abnormality, there may be a similar reduction in striatal ^{18}F-dopa uptake to that seen in CBD. PD has been extensively studied by PET, but although the changes in ^{18}F-dopa uptake have been striking, reported changes

TABLE 4 PET FINDINGS IN RELATED DISORDERS

Diagnosis	*Glucose/oxygen metabolism*	*^{18}F-Fluorodopa uptake*
Pick's disease (single case)	Frontal 49% normal Temporal, parietal and occipital normal[1]*	Not known
Alzheimer's disease	↓ Postparietal (L ± R)[2,3,4] ↓ Posttemporal[2,3,4] ↓ Premotor and parietal[5]	Slight ↓ putamen uptake[6] (in patients with rigidity only)
Parkinson's disease	↑ Basal ganglia[7] ↓ Medial frontal cortex[8]	Marked ↓ putamen uptake[9]
Steele-Richardson-Olszewski syndrome	↓ Frontal and global[10,11] ↓ Motor and premotor[12] ↓ Prefrontal[13] ↓ Frontal**[14]	↓ Caudate and putamen uptake[10,15]
Corticobasal degeneration	↓ Inferior parietal and thalamus[16] ↓ Superior temporal, inferior parietal, and occipital association cortex[17]	↓ Caudate, putamen and medial frontal cortex[17]

* After normalization to cerebellar values. ** After normalization to occipital cortex.

References [1] Kamo *et al.* (1987); [2] Frackowiak *et al.* (1981); [3] Foster *et al* (1984); [4] Foster *et al* (1986); [5] Haxby *et al.* (1988); [6] Tyrrell *et al.* (1989); [7] Wolfson *et al.* (1985); [8] Perlmutter and Raichle (1985), [9] Leenders *et al* (1989), [10] Leenders *et al* (1988). [11] Foster *et al.* (1988); [12] Goffinet *et al.* (1989), [13] D'Antona *et al* (1985), [14] Blin *et al* (1988); [15] Brooks *et al.* (1990), [16] Eidelberg *et al* (1989), [17] This study.

in oxygen or glucose metabolism have been subtle and trivial in comparison with the deficits seen in these patients.

Although recent computer visualization studies of midbrain cell loss in PD suggest that the disease preferentially destroys those midbrain dopaminergic neurons projecting to the striatum (German *et al.*, 1989), the finding of reduced regional tyrosine-hydroxylase activity in the ventral tegmental area of PD patients may indicate a lesion of the mesocortical dopamine system (Javoy-Agid and Agid, 1980).

PET studies have failed, however, to demonstrate any reduction in ^{18}F-dopa uptake into medial frontal cortex in either PD, or the other disorders listed in Table 4. In part, this may be due to the lower resolution of the PET cameras used in previous studies. The double-dissociation between PSP and CBD is of interest in this respect; while in PSP the most hypometabolic cortical areas are in the frontal lobe, the ^{18}F-dopa uptake into medial frontal cortex is normal. In contrast, in CBD patients we report a lesser involvement of frontal cortex oxygen metabolism, yet a significant reduction of ^{18}F-dopa uptake.

In CBD, the loss of midbrain neurons is most severe in the substantia nigra (Gibb *et al.*, 1989) and it may be that the reduction in medial frontal cortical dopamine uptake reflects a loss of the nigrocortical projection, rather than the ventral tegmental-cortical projection (Fallon, 1988).

We have studied patients with clinically diagnosed CBD at different stages of their disease and with a variable combination of clinical signs. The common findings were an asymmetric reduction of ^{18}F-dopa uptake into striatum, and reduced oxygen metabolism in superior temporal, inferior parietal cortex and occipital association cortex. Changes in frontal cortex were posterior and of lesser severity.

ACKNOWLEDGEMENTS

G.V.S. is supported by the Parkinson's Disease Society. We thank colleagues at the MRC Cyclotron Unit, Chemistry and Physics sections, whose expertise made these studies possible. We thank Ms C. J. V. Taylor and Mr G. Lewington for their help with scanning. We thank Professor P. K. Thomas, Dr N. J. Legg and Dr D. Jefferson for allowing their patients to be studied and Dr F. Scaravilli for performing the pathological examination of Case 1 and making the results available to us.

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(*Received November 3, 1989. Revised March 23, 1990. Accepted April 10, 1990*)

Brain (1991), **114**, 557–572

BLOOD-BRAIN BARRIER DAMAGE IN ACUTE MULTIPLE SCLEROSIS PLAQUES

AN IMMUNOCYTOLOGICAL STUDY

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SUMMARY

To investigate blood-barrier leakage of plasma proteins in acute plaques of multiple sclerosis (MS) the authors used immunocytological methods to examine frozen tissue removed at autopsy from recently active cases. Annular patterns of protein-rich leakage were seen which may help to elucidate the patterns observed using gadolinium-enhanced nuclear magnetic resonance imaging. Vessel wall damage was found in all acute plaques examined and this was associated with the intramural deposition of complement on smooth muscle components and with an infiltration of HLA-DR +ve macrophages. In addition, all acute cases examined had small plaques which contained particulate material within macrophages and astrocytes, on which complement and immunoglobulins colocated. Attempts to find similar material in cases of chronic MS, subacute sclerosing panencephalitis and in perivenous encephalomyelitis were unsuccessful. These results suggest that the inflammatory changes in early MS plaques may have some specificity which could be related to the antigens whose presence is inferred by the colocation of complement and immunoglobulin on material within activated macrophages and astrocytes.

INTRODUCTION

The observation that early plaques of multiple sclerosis (MS) usually develop around small cerebral blood vessels has long been considered to be an important clue to the pathogenesis of that disease (Reindfleisch, 1863; Dawson, 1916; Adams, 1989). This idea has recently received new impetus from gadolinium-enhanced nuclear magnetic resonance imaging (Gd MRI) which shows foci of blood-brain barrier (BBB) leakage as the earliest detectable change in the central nervous system (CNS) of patients with MS (Grossman *et al.*, 1988; Kermode *et al.*, 1988; Willoughby *et al.*, 1989). Furthermore, there is some indirect evidence that these foci are inflammatory in origin, raising the important suggestion that they may represent the primary lesion in MS (Miller *et al.*, 1988).

In this investigation we set out to study acute MS plaques employing immunocytological methods to examine frozen tissue removed at autopsy from recently active cases. First, a panel of antibodies specific for a variety of plasma proteins was used to assess vascular leakage within and around plaques. Does the distribution of protein leakage in tissue elucidate the patterns observed using Gd MRI (Kermode *et al.*, 1988)? Secondly, antibodies for a number of vascular structural components were used to look for evidence of structural abnormalities in leaking vessels. Finally, in conjunction with these reagents

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antibodies of known specificity for inflammatory cells were used in double labelling experiments to study the relationship between the three principal perivascular events in MS plaques, namely BBB leakage, inflammatory cell infiltration and demyelination.

Comparable studies were also carried out on tissue from cases of chronic active and inactive MS, subacute sclerosing panencephalitis (SSPE), acute perivenous demyelinating encephalitis (PVE), sagittal sinus thrombosis, cerebral neoplasm and normal cerebral white matter from road traffic accident cases without head injury.

METHODS

Tissues removed at autopsy from cases of acute MS and controls (Appendix 1) were snap frozen in liquid nitrogen and stored at −70° C. Most cases have already been documented in the literature (Appendix 1 references). Cryostat sections (8−10 μm) were air dried and fixed in acetone at 4° C for 5 min and reacted with various antibodies (Appendix 2) at 37° C for 30 min with gentle rotation in a humidity cabinet. All intermediate washes were in Tris or phosphate buffered saline (PBS) at pH 7.5.

Complement and immunoglobulin reagents gave consistent reactions in single and in double labelling experiments in glomeruli from known cases of systemic lupus erythematosus. Direct and indirect tests were successfully blocked using unlabelled reagents. The specificity of the monoclonal antibodies used has been largely documented in the literature (Appendix 2 references).

Sections reacted with fluorescein isothiocyanate (FITC) or tetramethyl rhodamine isothiocyanate (TRITC) labelled reagents were mounted in 'Citifluor' AF2 glycerol PBS and viewed with a 490 nm blue filter for FITC labels or a 546 nm green filter for TRITC labels. A polarizing filter for the detection of myelin birefringence (Adams, 1989) was used in conjunction with these filters.

Experiments employing fluorescent antibody techniques were duplicated using the peroxidase-antiperoxidase method (PAP). For the detection of antigens using primary rabbit antisera, acetone-fixed cryostat sections were pretreated with 1/50 normal swine serum. This was followed by the primary rabbit antibody at a dilution predetermined by titration to give the optimal reaction with the antigen to be detected. Swine antiserum to rabbit 1/100 was then applied followed by a PAP complex (Dako). Colour reactions were obtained using 3-amino-9-ethylcarbazole in N,N-dimethyl formamide and 0.3% hydrogen peroxide in water. Primary mouse monoclonal antibody was used in conjunction with normal rabbit serum. All reactions were at room temperature for 30 min and intermediate washes in PBS for 15 min. Sections were counterstained with haematoxylin and examined after covering with a water soluble mountant. Photographs were taken using a Leitz Ortholux 2 microscope fitted with a Wild MP 45/51 photomicrographic system, using Ektachrome 400 reversal film.

The data shown in the graphics (fig. 2, Advanced Color Technology System) were obtained by the systematic counting of vessels within and around acute MS plaques positive for abluminal fibrinogen and intracytoplasmic IgM using a micrometer grid system. Each ×40 field was viewed in sequence using (1) a polarizing filter for myelin birefringence, (2) a 490 nm blue filter for FITC labels and (3) a 546 nm green filter for TRITC labels. Negative controls were sections of histologically normal white matter and positive controls cases of SSPE and PVE (Cases 14−19 and 11−13, respectively, *see* Appendix 1). Demyelinating activity was assessed in the same section using the polarizing filter and in serial sections using conventional staining methods for myelin and myelin breakdown products (Luxol fast blue and oil red O). A rabbit antihuman Ig specific for myelin basic protein (MBP) was also employed to assess myelin lysis. Serial sections of the same plaques were also reacted with mouse monoclonal antihuman collagen IV, mouse monoclonal antihuman laminin or mouse monoclonal antiactin (HHF 35) to determine the distribution and extent of vessel wall abnormalities. Finally, the sections were washed in PBS, fixed in 10% formol saline, and stained by haematoxylin and eosin. Nuclear counts to further define the limits of the plaque were then obtained using an identical micrometer grid.

Serial sections of plaques analysed by these methods were also reacted with rabbit antihuman C3d-FITC. After washing in PBS, the macrophage specific monoclonal antibody Y1/82A was applied followed by rabbit antimouse Ig-TRITC. Vessels in these preparations showed abnormalities falling into 3 categories (fig. 5): (1) intramural complement reactions alone, (2) complement reactions accompanied by macrophage cuffing and (3) macrophage cuffing of vessels negative for complement. Using the micrometer the number of vessels in each category was counted in 20–25 ×40 fields in a series of contiguous transits parallel with the plaque edges which were defined using the polarizing filter for myelin birefringence. The sections were then washed and fixed in 10% formol saline and stained with haematoxylin and eosin. Nuclear counts were then made in a similar series of transits. Nuclear counts were made in normal white matter (Case 19) to provide a normal distribution for comparison with periplaque tissue. The data collected by these methods were used to construct graphs (e.g., fig. 5).

RESULTS

Leakage of plasma proteins

Of the 23 acute MS plaques examined (Appendix 1) all showed heavy accretions of fibrinogen, C1q, C3 and α-2 macroglobulin on endothelial cell abluminal membranes, and in wide zones of perivascular parenchyma (fig. 1A, 1A′). Weaker reactions for IgG, IgA and IgM were found in similar locations. Leakage was most marked around capillaries (<15 μm) and the small veins of the venocapillary junctions (15–40 μm) as they were traced back in serial sections into the centre of the plaques. This had the effect of producing a well defined ring of plasma protein leakage extending deeply into normally myelinated white matter around the edge of plaques (fig. 2A′, B′).

The endothelial cells of the vessels within these areas contained many vesicles giving strongly positive reactions for fibrinogen, C1q, C3 and α-2 macroglobulin (fig. 1B, C, D). In contrast, normal white matter controls and chronic inactive MS plaques (Appendix 1, Cases 4, 7, 8, 16–19) showed reactions for plasma proteins which were entirely confined to the lumina of blood vessels. However, similar studies of tissues containing areas of demyelination and inflammation from cases of SSPE and PVE (Appendix 1, Cases 11–13) showed similar vascular abnormalities but these were diffusely distributed in the affected tissues. Thus plasma protein leakage and endothelial cell vesiculation in acute MS plaques was distinctive only in its annular distribution in the capillary beds around affected cerebral veins. The demyelinated hypercellular centres of most of the acute MS plaques contained a disorganized mass of extracellular fibrin and strong reactions for all the plasma proteins tested. Although endothelial debris showing positive reactivity to von Willebrand factor was occasionally identified in this zone, in no instance was there evidence of haemorrhage or necrosis. Endothelial cells of the vessels within the centre of plaques appeared to be intact and in marked contrast to the annular zone of leakage outside the plaque, rarely showed plasma protein positive vesiculation.

Vessel wall damage

Cerebral capillaries possess complex basement membranes consisting of a macromolecular meshwork sleeve between the abluminal membranes of endothelial cells and the foot processes of astrocytes (Ichimura *et al.*, 1984). The principal structural components of basement membranes are type IV collagen and the glycoprotein laminin (Kennedy *et al.*, 1986; Essner and Lin, 1988). Using antibodies specific for collagen IV and for laminin we were unable to detect any evidence of a structural defect in the basement membranes of the leaking capillaries in the normal white matter around acute plaques. However, double labelling with collagen IV or laminin antibodies, macrophage specific antisera (Y1/82A, EBM 11 or RFD 7) and antibody to human HLA-DR showed an infiltration of vessel walls in demyelinated plaque edges with HLA-DR +ve

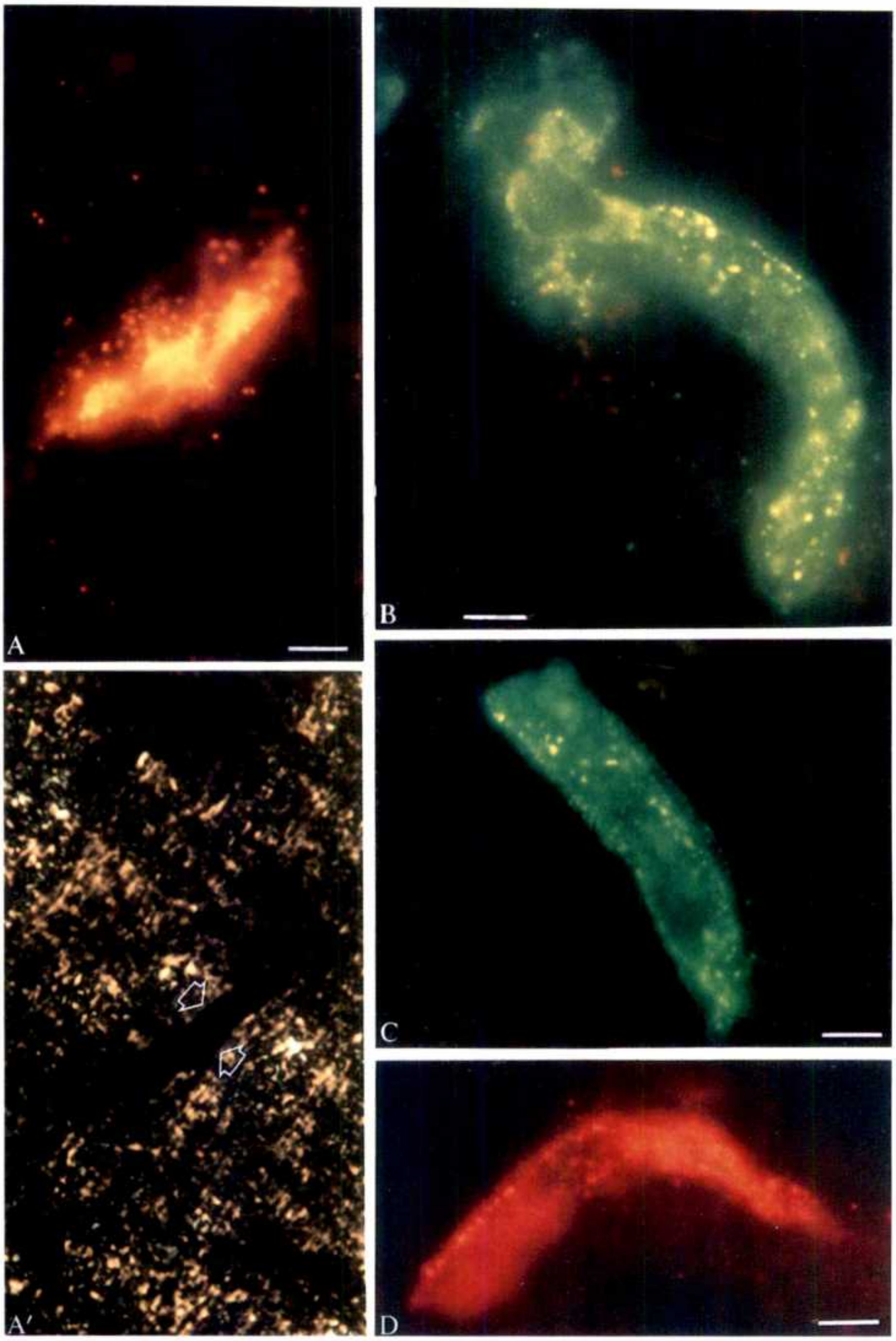

FIG. 1. Blood-brain barrier defects in periplaque normal white matter. A, fibrinogen leaking from a capillary (rabbit antihuman fibrinogen-TRITC) which, when observed with polarized light (A′), shows normal myelin birefringence up to the vessel walls (arrows). Bar = 10 μm. B, C, D, capillary endothelial cells containing vesicles positive for α-2 macroglobulin (FITC) (B), IgM (FITC) (C) and IgA (TRITC) (D). Bars = 5 μm.

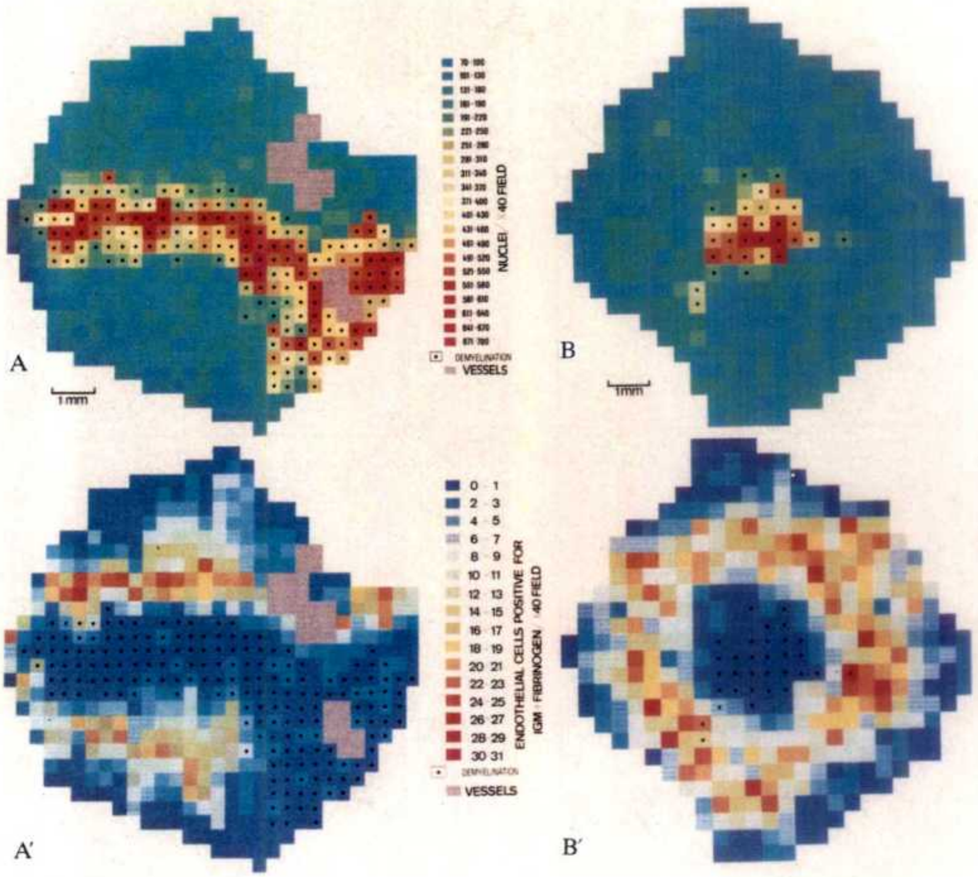

FIG. 2. Diagrams showing the distribution of BBB abnormalities associated with 2 acute plaques (A, B). The plaques are defined by their hypercellularity (nuclear counts; A, B) and by myelin lysis (dotted area). Both plaques (A', B') are surrounded by zones of perivascular fibrinogen leakage (e.g., fig. 1A) and endothelial cell endocytosis of IgM (e.g., fig. 1C) in normal white matter. (Advanced Color Technology System graphics; *see* Methods.)

macrophages, which was associated with dilatation, 'splitting' and reduplication of collagen IV and laminin positive structures (fig. 3C, D). In most of the acute plaques this process was most developed towards the plaque centres where the parenchyma was littered with collagen IV and laminin positive fragments (fig. 3C). Central veins in these plaques characteristically showed considerable connective tissue reduplication (fig. 3D). These vascular abnormalities were always associated with the presence of HLA-DR +ve macrophages, but areas of active demyelination in PVE and in SSPE did not show this effect (fig. 3A, B). Inactive plaques showed no connective tissue fragmentation in the parenchyma but within these lesions vessels showed collagenous thickening of their walls without evidence of plasma protein leakage. On the other hand, small leaking vessels located in plaque margins and showing partial perivascular myelin lysis always

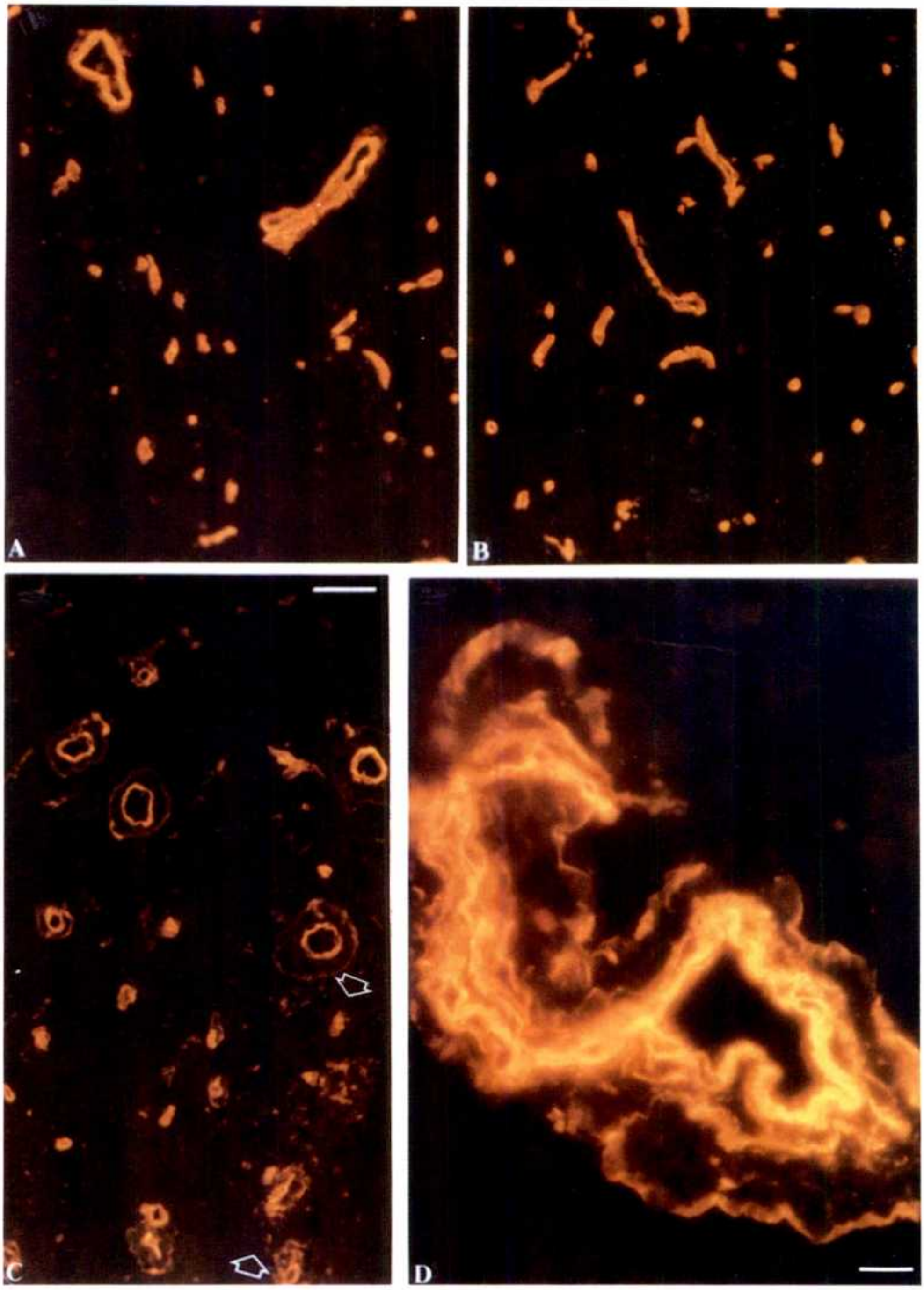

FIG. 3. Collagen type IV labelling of vessel walls (mouse monoclonal-TRITC) in demyelinated areas of SSPE (A) and PVE (B). C, similar labelling of the centre of an acute MS plaque. Vessel walls are dilated and the plaque parenchyma is littered with collagen IV positive fragments (arrows). Bar = 50 μm. A central vein of the same plaque (D) shows dilatation, fragmentation and apparent reduplication of collagen IV structures. Bars = 5 μm.

contained HLA-DR +ve macrophages but did not show connective tissue changes.

Cerebral venules frequently contain smooth muscle elements (Roggendorf and Cervós-Navarro, 1983). Using the monoclonal antibody HHF 35 which has specificity for actin we consistently found that many small vessels within leaking plaque margins were positive for this component (fig. 4c). However, vessels of comparable size and morphology within the demyelinated edge of acute plaques showed only weak reactions which were undetectable in plaque centres. A strikingly similar pattern was noticed in the distribution of reactions to C3d and to C9 within vessel walls (figs 4A, B, 5 inset). Double labelling confirmed that these intramural complement reactions were located on actin positive structures. Vessels in this area failing to react for actin showed little or no localized complement deposition. Vessels positive for actin in normal white matter both in controls and in nonleaking areas of MS brain were negative for C3d.

Double labelling with C3d and macrophage antibodies (fig. 4B, B′) now revealed that as the plaque margin was approached from surrounding normal white matter and vessels showed increasing cuffing with macrophages accompanied by connective tissue disruption, the intramural complement reactions became progressively weaker (fig. 5, inset) until in many cases they were not detectable in the plaque centre. An analysis of these changes is shown in fig. 5.

C3d deposition was also found on actin positive elements in leaking areas in PVE but we have been unable so far to detect this effect in 2 cases of SSPE.

Double labelling of MS plaques with C3d and IgG, IgA and IgM using direct and indirect techniques with primary antibody up to 1/10 failed to show any association between these C3d reactions and immunoglobulins in vessel walls despite the ease of detection of the latter in adjacent perivascular plasma cells and in some endothelial cells.

Using direct and indirect fluorescent antibody methods and the PAP technique with concentrations of primary antibody up to 1/10, we were unable to detect any significant reactions on myelin sheaths or on neuroglia for C3, C3c, C3d, C1q, IgM, IgG or IgA in or around MS plaques.

Colocation of complement and immunoglobulins

All the acute MS cases examined had some plaques containing particulate material strongly positive for C3d and for C9 which was chiefly located in cytoplasmic aggregates within HLA-DR +ve, Y1/82A and EBM 11+ve perivascular macrophages (fig. 6A, A′). In some plaques similar material was also detected in the cytoplasm of GFAP +ve astroctyes (fig. 6E) and in cells with the morphology of microglia showing positive reactions for Y1/82A and EBM 11. Similar granular and particulate material was occasionally seen scattered haphazardly in vessel walls and on capillary basement membranes (fig. 6A). In contrast to the complement reactions in vessels positive for actin described above, this material consistently showed reactions for IgG and IgM, although not for IgA. Moreover, in double labelling experiments there was a clear colocation between these immunoglobulins and C3d (fig. 6B, B′). Adjacent plaques within the same section of brain or spinal cord showed considerable variation in the amount and distribution of these complexes. They were rare in the largest plaques examined but were particularly associated with the smallest and most active lesions (less than 2 mm in diameter) where macrophages and astrocytes containing complexes tended to be irregularly distributed around and within the edges of the plaque (fig. 6E). Complexes

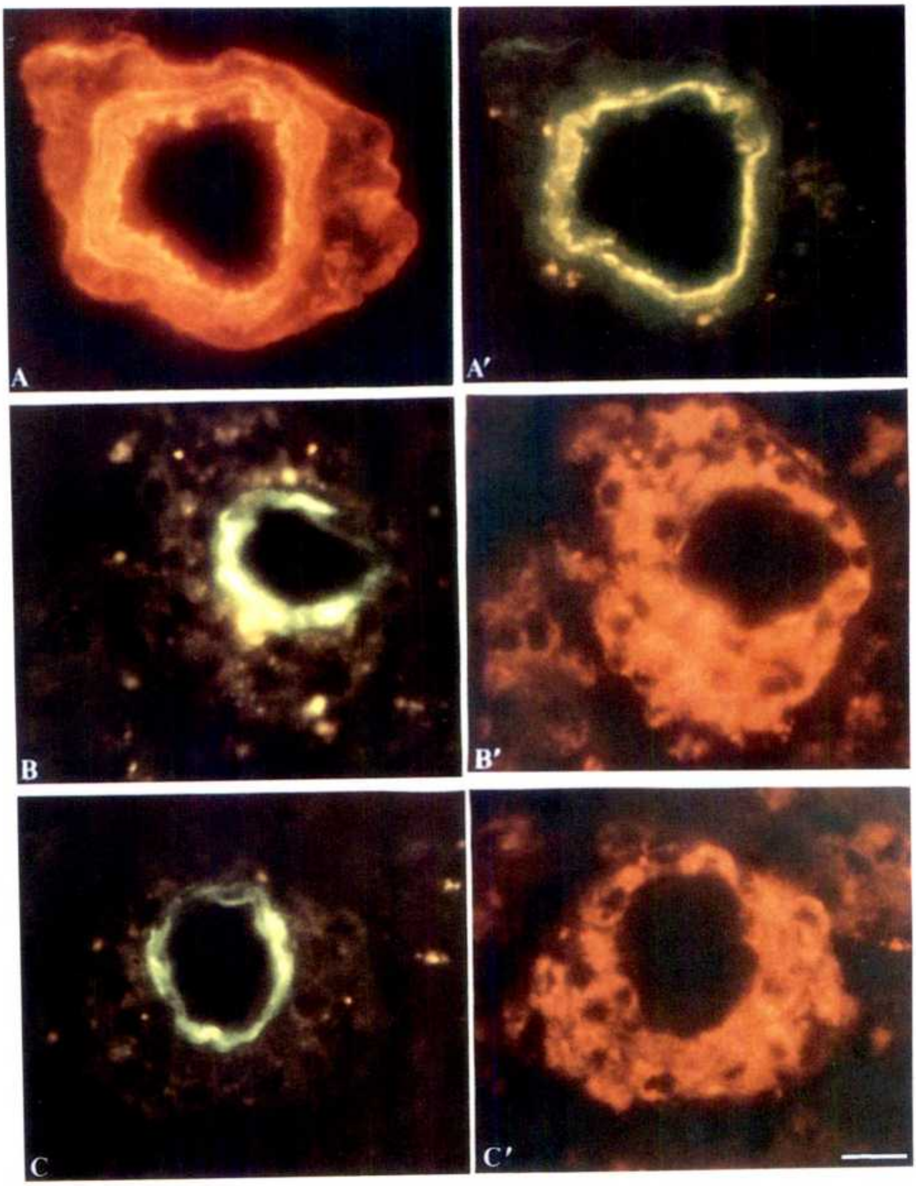

FIG. 4. Double labelling experiments on vessels within acute plaques. A, A′, dilatation, splitting and apparent reduplication of collagen type IV positive vessel wall structures (A, mouse monoclonal-TRITC) in which there is a localized reaction for C3d (A′, anti C3d-FITC). B, B′, characteristic localization of C3d in vessel wall structures (B, anti C3d-FITC) which are heavily cuffed with cells reactive for the panmacrophage monoclonal antibody Y1/82A (TRITC) (B′). C, C′, serial section of B and B′ showing reaction for actin (HHF 35-FITC) in the same distribution as C3d (C). The vessel is also labelled with the panmacrophage monoclonal EBM 11 (C′). Bar = 10 μm.

were not found in chronic or inactive plaques nor were they detected in control material including cases of SSPE in which a large amount of measles antigen was detected in neurons and in oligodendrocytes but not in macrophages. Complexes within macrophages

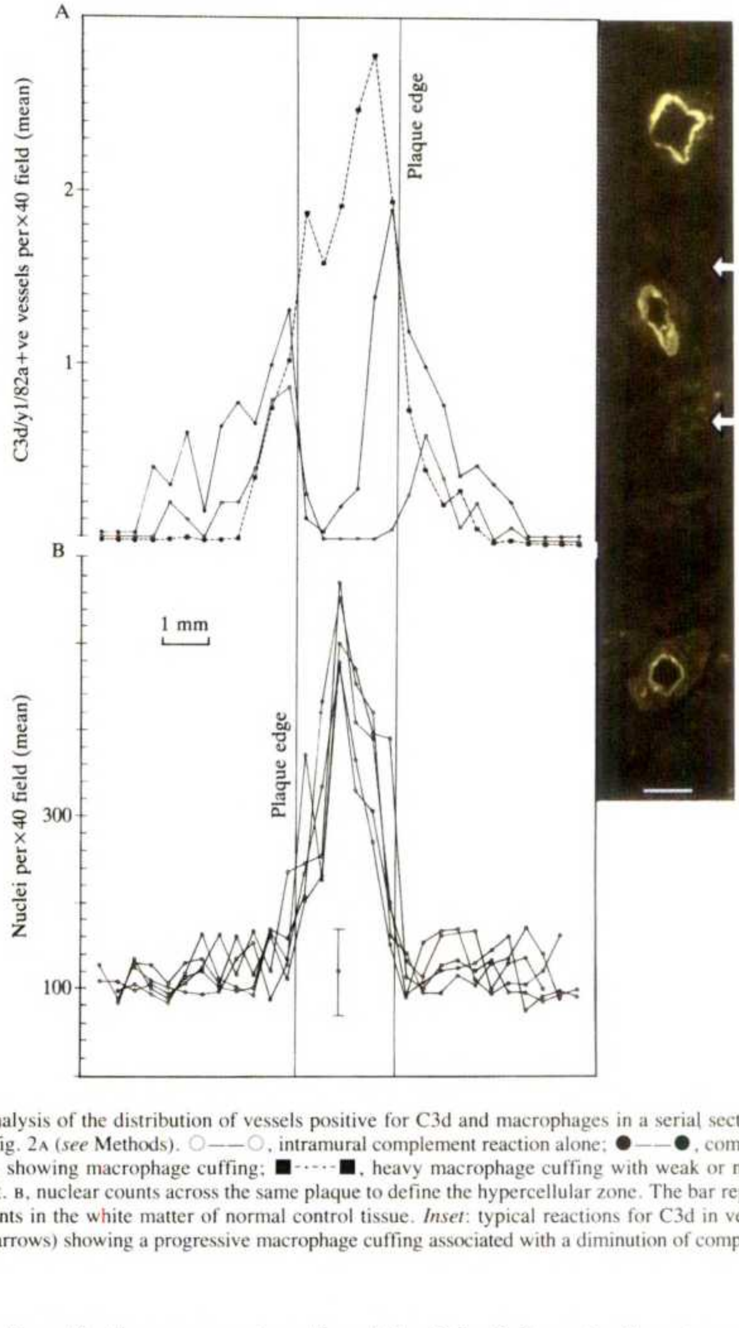

FIG. 5. A, analysis of the distribution of vessels positive for C3d and macrophages in a serial section of the plaque represented in fig. 2A (*see* Methods). ○——○, intramural complement reaction alone; ●——●, complement reactions in vessel walls, showing macrophage cuffing; ■·····■, heavy macrophage cuffing with weak or negative reactions for complement. B, nuclear counts across the same plaque to define the hypercellular zone. The bar represents the SEM for nuclear counts in the white matter of normal control tissue. *Inset*: typical reactions for C3d in vessel walls across a plaque edge (arrows) showing a progressive macrophage cuffing associated with a diminution of complement reactivity. Bar = 50 μm.

in MS were found both as aggregates of particles 0.3–0.9 μm in diameter and associated with the membranes of intracytoplasmic vacuoles (fig. 6D, F). Although reactions to collagen IV, laminin, actin and MBP were occasionally detected in macrophages containing C3d/IgG complexes, in no instance did these colocate with complement or immunoglobulin in double labelling experiments. Complement positive complexes were

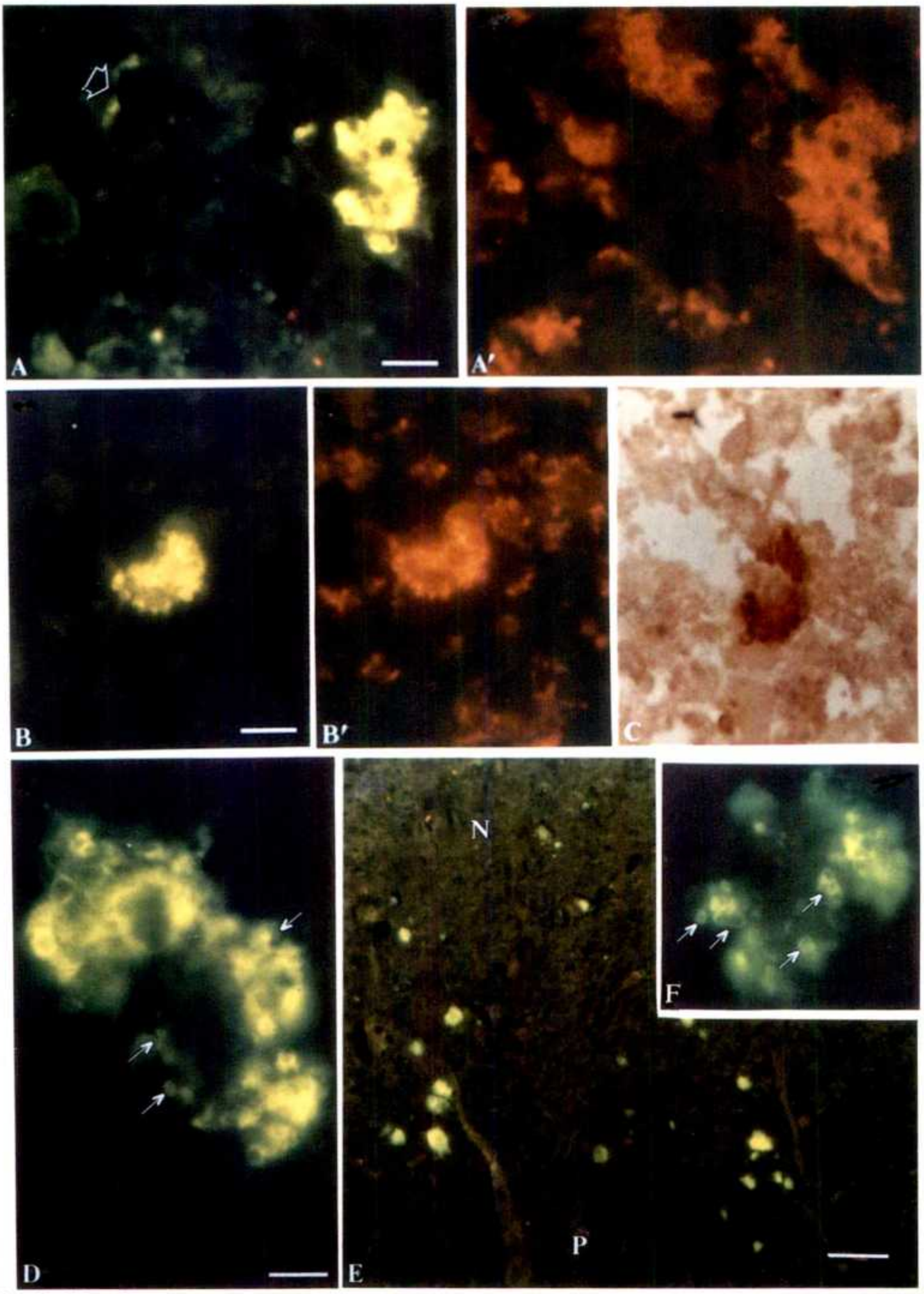

FIG. 6. Colocation of complement and immunoglobulin in acute MS plaques. A, A′, double labelling for C3d-FITC (A) and Y1/82A-TRITC (A′) locates complement within macrophages. C3d positive material is also seen in the wall of a small vessel (arrow). Bar = 10 μm. B, B′, double labelling for C3d-FITC (B) and IgG-TRITC (B′) shows the colocation of complement and immunoglobulin within a macrophage. C, peroxidase-antiperoxidase for C3d. Macrophage showing positive vacuoles and particles. D, F, C3d-FITC showing groups of intracellular complement coated particles (arrows) within macrophages. Bar = 5 μm. E, small hyperactive plaque (P) with C3d positive macrophages and astrocytes scattered throughout the plaque margin. N = normal white matter. Bars = 100 μm.

not birefringent nor did they stain with Luxol fast blue. Particles in capillary basement membranes and in the parenchyma showed similar reactions and morphology (fig. 6A). Macrophages containing C3d/IgG complexes were also found in the meninges in some of the acute MS cases.

DISCUSSION

What do our results contribute to the interpretation of MR images? Barnes *et al.* (1987) have shown experimentally that a vasogenic protein-rich oedema is characterized by elevations of T_1 and T_2, but T_2 is relatively less elevated when compared with a protein-free oedema. This produces a high T_1/T_2 ratio and this has been recorded in gadolinium-enhancing lesions in MS (Miller *et al.*, 1988). We have shown a considerable leakage of large plasma proteins from the capillary beds and venocapillary junctions around all the acute plaques examined. Moreover, this takes the form of an annulus around the plaque edge, a feature described by Grossman *et al.* (1988) and by Miller *et al.* (1988) in enhancing lesions. The hypercellular centres of our acute plaques consistently showed markedly less endothelial vesiculation than was seen at the plaque edges. Excessive micropinocytosis is a well documented feature of BBB disturbance (Mchedlishvili *et al.*, 1986) and has been described in MS biopsies by Brown (1978). The vascular connective tissue changes in this region strongly suggest a process of organization and repair. Taken together these features may account for the acute lesions described by Kermode *et al.* (1988) in which gadolinium leakage is maximal at the plaque periphery and reduced in the plaque centre. The appearance of considerable protein leakage in normally myelinated areas around plaques suggests that leakage per se is not necessarily demyelinating. We found similar large areas of protein leakage in wide areas of tissue apparently unassociated with plaques, a finding confirmed in formalin fixed tissue from recently active cases (C. W. M. Adams, personal communication). This would suggest that MR images may occur in the absence of any demyelinating lesion.

What is the significance of the structural changes observed in the blood vessels of the plaque? Excessive vascular connective tissue and the loss of smooth muscle elements are closely associated with the presence of activated DR +ve macrophages and are confined to demyelinated zones. Activated macrophages possess C3b receptors and the process observed in cerebral vessels in which these cells were closely applied to C3d coated actin (e.g., fig. 4c, c′) may be a complement-mediated immune attack. This does not necessarily mean that vessel wall components such as actin are a specific target or that complement deposition is a necessary precursor to vessel wall damage. The appearance of C3d in vessel walls in PVE as well as MS suggests rather that complement deposition in this location is likely to be a secondary consequence of inflammation though probably not without pathological significance in the inflammatory changes in vessel walls which appears to be a major feature of the acute MS plaque. The fact that the intramural complement deposits were not colocated with immunoglobulin suggests that at this location complement deposition resulted from alternate pathway activation. In agreement with our finding of vascular deposits of complement in MS, Compston *et al.* (1989) have recently described granular deposits of C9 and the terminal complement complex in capillary endothelial cells in MS plaques and adjacent white matter. They also found similar deposits in SSPE.

What light do our results shed on the relationship between vascular leakage, cellular infiltration and demyelination? Approaching the margins of acute plaques, demyelination was first detected in periplaque tissue as a reduction in birefringence around small blood vessels cuffed with intramural DR +ve macrophages in the absence of any vascular connective tissue abnormality. Histological evidence suggests (Adams, 1989) and MRI confirms (Kermode *et al.*, 1988) that many plaques grow by centrifugal expansion of their edges. It is therefore possible that an inflammatory cell microvasculitis precedes demyelination at the margins of lesions. This is supported by our observation that similar microvascular foci occur in recently active cases apparently unassociated with established plaques. These lesions, while showing considerable plasma protein leakage and endothelial cell vesiculation, had no vascular connective tissue abnormalities. These small and apparently isolated perivascular foci may represent an early event in the evolution of a plaque, but they do not point to a primary attack on the vessel wall itself.

Our failure to demonstrate complement or immunoglobulin deposition on myelin or on oligodendrocytes in our material was unexpected. Myelin fragments activate the alternate and classical complement pathways in vitro (Vanguri *et al.*, 1982) and C3d-IgM complexes have been demonstrated on myelin sheaths in the demyelinating peripheral neuropathies (Hays *et al.*, 1988). Our results argue against specific humoral immune-mediated damage to myelin in MS and support the idea (Wisniewski and Bloom, 1975; Adams, 1989) that myelin may be damaged as an innocent bystander rather than as a specific target in the inflammatory reactions which develop around and within the walls of cerebral vessels. Compston *et al.* (1989) likewise failed to find complement deposits on myelin sheaths in or around MS plaques.

When complement and immunoglobulin are detected at the same location in tissues or on micro-organisms this provides evidence that antibody has reacted with antigen and that complement has been fixed by the classical pathway. Our finding in acute MS of particles coated with C3d, C9, IgG and IgM within HLA-DR +ve macrophages and in vessel walls reinforces this evidence and suggests that an antigen within these complexes may be the initiator of the activation process leading to immune adhesion and phagocytosis. Of the various inflammatory conditions examined in this study MS was the only one that showed this feature. Immune complexes have been detected in MS CSF (Coyle, 1987) but their pathological significance has remained speculative. It may not be assumed that the finding of complexed material in lesions is certain evidence that the complexes are the cause of the damage, but a primary pathological role for the complexes described here is suggested by their exclusive association with acute plaques and their disposition within HLA-DR +ve macrophages in the advancing borders of small hyperactive lesions. The presence of IgM in these complexes further supports this acute association. An obvious priority is to identify the particulate material on which complement and immunoglobulin colocates in small acute lesions.

ACKNOWLEDGEMENTS

We thank Dr M. L. Rossie for supplying material from Case 1, Dr M. L. Cuzner for Cases 3–5, and Dr R. Poston for Case 6. The late Professor C. W. M. Adams kindly provided Case 10. Dr K. C. Gatter supplied Y1/82A antibody, Professor G. Janossy RFD 7 and Professor J. O'D. McGee EBM 11. We thank the staff at the department of mathematics of Essex University for the production of graphics, and Dr S. Milne and her staff for laboratory facilities at Colchester General Hospital. Professor George Dick provided support and encouragement throughout. The work was supported by grants from the Multiple Sclerosis Society of Great Britain and Northern Ireland, Action for Research into Multiple Sclerosis, the Glenmore Trust, Edinburgh, and in part from the Medical Research Council.

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(*Received February 27, 1990. Accepted April 10, 1990*)

APPENDIX 1. AUTOPSY MATERIAL EXAMINED

Case	*Age (yrs), sex*	*Clinical diagnosis*	*Total duration*	*Duration of terminal illness*	*Frozen blocks*	*Histology*	*Previous published data in case*	*No. of plaques*
1	26, F	Acute MS	4 mos	2 mos	W69 A W69 B W69/1 W69/2† W69/3†	Acute fulminating MS	Esiri and Reading (1987), Case 1	10
2	52, F	Acute MS	2 yrs	1 mo.	BM 1†	Acute MS	Esiri and Reading (1987), Case 2	5
3	26, M	Acute MS	2 yrs	Not known	C316†	Acute MS	Woodroofe *et al.* (1986)	1
4	33,	Progressive MS	10 yrs	6 mos	Q7 (B260)	Chronic active	–	1
5	35, M	Acute MS	6 mos	1 wk	B285†	Acute MS	–	3
6	43, F	Acute MS	2 yrs	6 mos	B 30†	Acute MS	–	1
7	61, F	Chronic inactive MS	43 yrs	6 mos	B8946	Inactive MS	Esiri and Reading (1987), Case 7	2
8	65, F	Chronic inactive MS	45 yrs	2 mos	9055	Inactive MS	Esiri and Reading (1987), Case 8	1
9	51, M	Chronic active MS	20 yrs	–	B8672	Border active	Esiri and Reading (1987), Case 6	1
10	60, M	Acute MS	26 wks	26 wks	G112/85*†	Acute MS	Adams *et al.* (1990), Case A	3
11	15, M	SSPE	12 mos	6 mos	C398/a C398/b		Esiri *et al.* (1982)	
12	18, F	SSPE	13 yrs	–	C1000		Esiri *et al.* (1982)	
13	20, M	Acute encephalitis	–	2 wks	C645/a C645/b C645/c	Perivenous encephalomyelitis	Esiri *et al.* (1989)	
14		Sagittal sinus thrombosis			C1226	Normal WM+adjacent infarct		
15		Cerebral neoplasm			C1087	Normal WM+adjacent necrosis		
16		RTA			C1231	Normal WM.No cerebral injury		
17		RTA			C1229			
18		RTA			C1248			
19		RTA			C1225			

* Paraffin block, † C3d/Ig complexes detected. RTA = road traffic accident. WM = white matter.

APPENDIX 2 MONOCLONAL AND POLYCLONAL ANTIBODIES EMPLOYED

Antibody to:	*Antigen source*	*Antibody source*	*Labels (when applicable)*	*Final working dilution*	*Source*
Fibrinogen	Human	Rabbit	FITC	1/200	Dako
Immunoglobulins	Rabbit	Swine	FITC or TRITC	1/100	Dako
Alpha-2 macroglobulin	Human	Rabbit	–	1/100	Dako
IgA, IgG, IgM	Human	Rabbit	–	1/250	Dako
C1q complement	Human	Rabbit	FITC	1/200	Dako
C3 complement	Human	Goat	–	1/100	Sigma
IgG	Goat	Rabbit	FITC	1/100	Sigma
C3c complement	Human	Rabbit	FITC	1/100	Dako
C3d complement	Human	Rabbit	FITC	1/100	Dako
C9 complement	Human	Rabbit	–	1/80	Serotec
IgG (gamma chains)	Human	Rabbit F(ab')$_2$	FITC or TRITC	1/250	Dako
IgA (alpha chains)	Human	Rabbit F(ab')$_2$	FITC or TRITC	1/250	Dako
IgM (mu chains)	Human	Rabbit F(ab')$_2$	FITC or TRITC	1/250	Dako
Collagen type IV	Human	Mouse m.c. CIV 22	–	1/50	Bionuclear, Dako
Laminin	Human	Mouse m.c.	–	1/20	Serotec
Immunoglobulins	Mouse	Rabbit	FITC or TRITC	1/100	Dako
Actin	Human	Mouse m.c. HHF 35	–	1/500	Tsukada *et al* (1987)
Macrophages	Human	Mouse m.c. Y1/82A	–	1/50	Davey *et al.* (1988)
Macrophages	Human	Mouse m.c. EBM 11	–	1/50	Franklin *et al* (1986)
Macrophages	Human	Mouse m.c. RFD 7	–	1/50	Janossy *et al.* (1986)
Glial fibrillary acidic protein	Human	Mouse m c. GF2	–	1/100	Dako
HLA-DR	Human	Mouse m.c. DK 22	–	1/50	Dako
von Willebrand factor	Human	Mouse m.c F8/86	–	1/50	Dako
Myelin basic protein	Human	Rabbit	–	1/20	Dako

Brain (1991), **114**, 573–583

CELLS SECRETING ANTI-MAG ANTIBODY OCCUR IN CEREBROSPINAL FLUID AND BONE MARROW IN PATIENTS WITH POLYNEUROPATHY ASSOCIATED WITH M COMPONENT

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SUMMARY

Occurrence and distribution of cells secreting antibodies against myelin associated glycoprotein (MAG) were studied in 9 patients with polyneuropathy associated with the monoclonal (M) component in serum. Utilizing an immunospot assay, we found that 4 of 7 patients with polyneuropathy associated with an IgM M component had cells secreting anti-MAG IgM antibody in cerebrospinal fluid (CSF) numbering between 1 per 212 and 1 per 3333 mononuclear cells. All 7 patients had cells secreting anti-MAG IgM antibody in bone marrow (median value 1 per 2000 cells). In contrast, peripheral blood from only 2 of these patients contained low numbers of such cells. One patient with polyneuropathy associated with an IgA M component had cells secreting anti-MAG IgA antibody in CSF, and 1 with an IgG M component had cells secreting anti-MAG IgG antibody in CSF; both patients also had anti-MAG IgM antibodies detectable in CSF only by ELISA. These 2 patients may thus have concurrent intrathecal production of antibodies of 2 different isotypes which are directed against the same or different epitopes of MAG. The production of antibodies directed against a component of myelin occurring in the immediate vicinity of the peripheral nervous system might be involved in the pathogenesis of the polyneuropathy.

INTRODUCTION

Polyneuropathy and monoclonal gammopathies are associated (Iwashita *et al.*, 1974; Kahn *et al.*, 1980), and a causal relationship between the presence of IgM M component and polyneuropathy has been suggested (Forssman *et al.*, 1973; Latov *et al.*, 1980, 1981; Dalakas and Engel, 1981; Melmed *et al.*, 1983; Nobile-Orazio *et al.*, 1983; Kelly, 1985). In about 50% of patients with IgM M components in serum, the IgM has been shown to bind to myelin components, mainly myelin associated glycoprotein (MAG) (Latov *et al.*, 1981; Braun *et al.*, 1982; Melmed *et al.*, 1983; Steck *et al.*, 1983; Kelly, 1985), glycolipids (Ilyas *et al.*, 1984; Chou *et al.*, 1985; Miyatani *et al.*, 1987), a 25 kDa peripheral nerve myelin protein (Nobile-Orazio *et al.*, 1984; O'Shannessy *et al.*, 1986; Cruz *et al.*, 1988), and certain glycosphingolipids (McGinnis *et al.*, 1988). Direct injection of M component sera together with complement into nerves of suitable species induces demyelination (Hays *et al.*, 1987; Willison *et al.*, 1988), but systemic injection does not produce disease despite long-term observation and treatment (Steck *et al.*, 1985).

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The pathogenetic role of antibodies against nervous tissue remains unsettled. About 50% of patients with polyneuropathy and an IgM M component have no antibody activity in serum against myelin components.

In nerve biopsies (Meier *et al.*, 1983; Smith *et al.*, 1983; Stefansson *et al.*, 1983) and in skin biopsies containing nerve fibres (Jønsson *et al.*, 1987), deposits of IgM have been shown. Thus IgM antibodies of putative pathogenetic importance must in some way have reached the endoneurial space in spite of a blood-nerve barrier which is normally impermeable to such large molecules (Olsson and Reese, 1971). Theoretically, 3 possible different mechanisms could be envisaged for this. First, a minute but long-term leakage of IgM to the endoneurial space; secondly, damage to the blood-nerve barrier by other means, for example T cell-mediated inflammation; and thirdly, as a consequence of local antibody production.

We have recently described the presence of anti-MAG antibodies of the IgM isotype in cerebrospinal fluid (CSF) from 13 consecutive patients with polyneuropathy associated with IgM M component, in some of the patients even when such antibodies were not detectable in serum (Cruz *et al.*, 1991). It thus seems that anti-MAG antibodies are common in CSF in patients with polyneuropathy associated with IgM M component, and that they may in fact be produced intrathecally.

In this report, we study the distribution of the anti-MAG specific B cell response in patients with polyneuropathy associated with M component, by enumerating cells that secrete anti-MAG antibodies of different isotypes in blood, CSF and bone marrow. Our data give further evidence for an anti-MAG antibody response which is also localized intrathecally in patients with polyneuropathy associated with M component.

MATERIAL AND METHODS

Patients

CSF, blood and bone marrow samples were obtained from 9 selected patients (6 females) with chronic sensorimotor polyneuropathy, a monoclonal component in serum and anti-MAG IgM antibodies in serum and/or CSF as determined by ELISA (*see below*). The age of the patients was 59–79 yrs (mean 71; Table 1). Serum electrophoresis and immunofixation had revealed M component of IgM isotype in 7 patients, IgG isotype in 1 patient and IgA isotype in the remaining 1 (Table 1). Case 2 fulfilled the criteria for Waldenström's macroglobulinaemia, while the other 8 patients belonged to the entity of monoclonal gammopathy of unknown significance (MGUS). Case 8 had cobalamin deficiency as a putative additional cause of his polyneuropathy. Moderate to severe reduction in nerve conduction velocity was present in all patients except Case 3 in whom neurophysiological signs of denervation were prominent. Neurophysiological investigation was, however, not done in Case 2.

Control bone marrow cells were obtained from 7 patients undergoing orthopaedic surgery. These patients had no other known disease. Their age was 28–52 (mean 38) yrs.

Sixty subjects (38 females) who suffered from 'tension' headache and had no evidence of organic neurological disease, constituted our 'normal' controls for anti-MAG IgM values in CSF and serum measured by ELISA. Their age was 20–68 (mean 43) yrs. All subjects had normal findings on routine CSF examinations.

Routine CSF studies

A survey of CSF and serum variables is presented in Table 1. All 9 patients with polyneuropathy and M component in serum had normal mononuclear cell counts in CSF. The cell-free CSF supernatant obtained after centrifugation and corresponding serum were analysed for albumin and IgG concentrations by immunoprecipitation nephelometry, and for IgA and IgM levels by ELISA (Lolli *et al.*, 1989). Five of

TABLE 1 ISOTYPE OF THE M COMPONENT, CSF AND SERUM FINDINGS IN 9 PATIENTS WITH POLYNEUROPATHY AND SERUM M COMPONENT*

					Ig concentrations						
					IgG		*IgA*		*IgM*		
Case	*Age (yrs)/ sex*	*Isotype and light chain type of M component in*	*Mononuclear cells in CSF (5×10⁶/l)*	*CSF/serum albumin ratio (9.2)*	*CSF (38 mg/l)*	*Serum (14 g/l)*	*CSF (3.0 mg/l)*	*Serum (3.7 g/l)*	*CSF (0.4 mg/l)*	*Serum (2.3 g/l)*	*IgM[1] index (0.06)*
1	65 M	Mκ	1	19.6	117	12	16.0	0.8	11.0	21.7	0.03
2	79 F	Mκ	2	11.6	85	12	3.5	1.7	5.6	19.6	0.03
3	78 F	Mλ	1	4.5	9	5	5.5	4.0	8.2	17.0	0.11
4	79 F	Mκ	1	26.7	178	12	0.6	0.5	36.0	13.7	0.10
5	60 F	Mκ	1	6.5	33	9	0.5	0.6	1.9	12.0	0.02
6	69 M	Mκ	1	9.6	25	5	6.0	0.6	15.0	27.5	0.06
7	79 F	Mκ	1	11.2	39	7	0.4	0.2	27.0	4.0	0.60
8	71 F	Aκ	2	6.4	20	6	8.9	24.4	1.1	0.7	0.25
9	59 M	Gλ	1	5.6	53	19	4.4	3.8	1.0	0.8	0.28

* Numbers within brackets denote upper reference values. [1] IgM index = (CSF IgM/serum IgM):(CSF albumin/serum albumin)

the patients (Cases 1, 2, 4, 6, 7) had increased CSF/serum albumin ratio (Tibbling *et al.*, 1977) as sign of damage to blood-brain barrier or blood-nerve barrier in the area of nerve roots. CSF IgG index equal to (CSF IgG/serum IgG):(CSF albumin/serum albumin) (Link and Tibbling, 1977) was normal (≤ 0.7) in all patients, while 1 patient (Case 6) had elevated IgA index (>0.34) and 5 had increased IgM index (>0.06), reflecting intrathecal production of corresponding immunoglobulins. For demonstration of oligoclonal IgG bands, we examined unconcentrated CSF and corresponding diluted plasma by agarose isoelectric focusing followed by transfer of separated proteins to nitrocellulose membrane, immunolabelling, avidin-biotin amplification and peroxidase staining (Olsson *et al.*, 1984). Only the patient with M component of IgG isotype (Case 9; Table 1) had, as expected, regularly spread IgG bands in both CSF and plasma.

Preparation of MAG

MAG was prepared by lithium diiodosalicylatephenol method (Quarles *et al.*, 1983) from bovine brain myelin (Norton and Poduslo, 1973). Purity of MAG was defined by SDS electrophoresis (Laemmli, 1970) which showed a single band migrating at 100 kDa, and by Western blot (*see below*) using serum from a patient with known monoclonal anti-MAG IgM which bound to the single 100 kDa band.

ELISA for measurement of anti-MAG IgM antibodies

Ninety-six well polystyrene microtitre plates (Dynatech-M129B; Dynatech, Alexandria, USA) were coated overnight at 4° C with 100 μl aliquots containing 1 μg/ml of purified MAG dissolved in phosphate buffered saline (PBS) at pH 7.4, and thereafter washed 5 times in PBS containing 0.05% Tween 20. Wells were blocked with 200 μl/well of PBS containing 2% bovine serum albumin (BSA) (Sigma, St Louis, USA); 100 μl of serum and CSF adjusted to 1 μg/ml of IgM were incubated overnight at 4° C. This IgM concentration yielded optimal discrimination between a positive control consisting of serum from a patient with polyneuropathy and known high levels of anti-MAG IgM antibodies in serum, and a negative control consisting of pooled normal serum. CSF samples with IgM concentrations less than 1 μg/ml were applied undiluted except for the addition of 20 μl of PBS per 100 μl of CSF. After washing, alkaline phosphatase-conjugated rabbit antihuman, μ-chain antiserum (Dako, Copenhagen, Denmark), diluted 1/2000 was added. After incubation at room temperature for 2 h, plates were washed and incubated with 100 μl per well of substrate p-nitrophenylphosphate (1 mg/ml; Sigma) in 10% diethanolamine and 0.02% MgCl buffer, pH 9.8. Enzyme reaction was developed at room temperature until absorbance value between 1.1 and 1.2 was reached for the positive control. Pooled normal serum never gave an absorbance value above 0.08. Plates were read in an automated ELISA spectrophotometer at 405 nm. Samples were considered positive when the absorbance value was higher than +3 SD of the mean value obtained from analysis of sera and CSF from 60 patients with tension headache. Range of absorbance values for these sera was 0–0.10 (mean 0.023, SD 0.027) and for CSF 0–0.077 (mean 0.016, SD 0.022), yielding mean values +3 SD of 0.11 for serum and 0.083 for CSF.

Western blot analysis

To confirm the presence of anti-MAG antibodies, all sera were examined by Western blotting. MAG was subjected to SDS electrophoresis in 12.5% polyacrylamide slab gel (Laemmli, 1970) followed by transfer of MAG to nitrocellulose membranes (0.45 μm pore size; Schleicher and Schüll, Kassel, Germany) (Towbin *et al.*, 1979). The nitrocellulose membranes containing MAG were then cut into 4.5 mm strips and incubated for 1 h in PBS containing 5% BSA to avoid nonspecific binding. Nitrocellulose strips were then incubated with 1/100 diluted patient's serum overnight at room temperature on a shaker platform. After several washes in PBS, strips were incubated with rabbit antihuman IgM, μ-chain specific (Dako) diluted 1/500, followed by incubation with 1/500 diluted biotinylated goat antirabbit IgG antiserum and 1/200 diluted avidin-biotin peroxidase complex (ABC Vectastain Kit, Vector Lab., Burlingame, USA) (Olsson *et al.*, 1984). After washing, the reaction was developed according to Kaplow (1975), followed by washing and drying.

Sampling and isolation of mononuclear cells from CSF, blood and bone marrow for nitrocellulose immunospot assay

Twenty ml samples of CSF were collected in siliconized glass tubes. CSF cells were counted by phase contrast microscopy. Specimens with erythrocyte counts $>50\times10^6$/l were excluded. Immediately after sampling, CSF was centrifuged at 1000 rpm for 10 min. CSF was discarded and cells (CSF-L) were resuspended in tissue culture medium consisting of Iscove's modification of Dulbecco's medium (Flow

Laboratories, Irvine, Scotland) with 2 mmol L-glutamine (Flow), 5% (v/v) fetal calf serum (Gibco, Paisley, UK) and antibiotics. After centrifugation and 2 additional washings followed by centrifugation, the CSF-L were rediluted to $4-16\times10^4$ cells/ml. The total time from lumbar puncture to application of cells into microtitre plate wells used for the immunospot assay (*see below*) was less than 1 h. Peripheral blood was taken simultaneously in heparinized tubes, and lymphocytes (PBL) were immediately separated by density gradient centrifugation on Ficoll (Lymphoprep, Nygaard, Oslo, Norway), washed 3 times in PBS, counted and adjusted to 10^6 cells/ml. Bone marrow aspirates were obtained from the iliac crest. These cells (BML) were treated as for PBL.

Nitrocellulose immunospot assay

To enumerate cells secreting anti-MAG antibodies of IgG, IgA and IgM isotypes, or cells secreting IgG, IgA or IgM immunoglobulins among isolated CSF-L, PBL or BML, we employed a solid-phase enzyme-linked immunospot assay and used microtitre plates with 96 wells and lined with nitrocellulose (Millititre-HA, Millipore Co., Bedford, USA) as described (Baig *et al.*, 1989; Olsson *et al.*, 1990). Coating of nitrocellulose was performed overnight at 4° C with 100 μl aliquots per well of a solution containing 10 μg/ml of our MAG preparation (*see* Preparation of MAG) for enumeration of cells secreting anti-MAG antibodies, or with 100 μl of diluted affinity-purified goat antihuman heavy chain specific IgG, IgA or IgM antiserum (Sigma) for parallel enumeration of total numbers of IgG, IgA or IgM secreting cells. Optimal concentrations for coating have previously been established. Coating solutions were removed by suction through the nitrocellulose membranes, and the plates were washed with PBS. 100 μl aliquots containing 10^5 PBL, 10^5 BML or $4-10\times10^3$ CSF-L were placed in individual wells. Mostly, numbers of CSF-L were insufficient for application into wells other than those coated with MAG. After incubation overnight at 37° C in humidified atmosphere and 7% CO_2, wells were emptied and washed. 100 μl of diluted high-affinity purified biotinylated goat antihuman IgG, IgA or IgM, heavy chain specific (Sigma) were added to appropriate wells followed by washing, incubation with diluted avidin-biotin peroxidase complex, washing and staining (*see* Western blot). Formed immunospots were counted using a dissecting microscope at a magnification of ×25 Values obtained were standardized to numbers of spots per 10^4 cultured cells. Experiments with inhibitors of protein synthesis and secretion have revealed that the immunospots reflect the production of antibodies (Zachau *et al.*, 1989).

Statistical analysis

The Mann-Whitney U test was used for statistical evaluation.

RESULTS

Anti-MAG antibodies detected by ELISA and Western blot

All 7 patients with polyneuropathy associated with IgM M component were positive in ELISA for anti-MAG IgM antibodies in CSF (Table 2). Six of these patients were clearly positive in corresponding serum for anti-MAG IgM antibodies, while 1 patient (Case 3) had a borderline value in serum. According to the ELISA results, only 2 patients (Cases 3, 5) had intrathecal anti-MAG IgM antibody production. Western blot performed on serum confirmed the presence of anti-MAG IgM antibodies in 6 of the 7 patients with IgM M components (Case 6 was negative) (Table 2).

An unexpected finding was that the patient with a polyneuropathy associated with an M component of IgA isotype and the patient with an M component of IgG isotype were both positive by ELISA for anti-MAG IgM antibodies. They were detected only in CSF and not in serum as evidence of intrathecal production. On the other hand, anti-MAG antibodies of the IgA or IgG isotypes were not demonstrable in serum or CSF from these 2 patients when examined by ELISA, or in serum when analysed by Western blot.

TABLE 2. ANTI-MAG IgM ANTIBODY REACTIVITY MEASURED BY ELISA AND WESTERN BLOT IN 9 PATIENTS WITH POLYNEUROPATHY AND SERUM M COMPONENT*

Case	*Isotype of M component in serum*	*Absorbance values in ELISA*		*Western blot of serum*
		Serum (0.11)	*CSF (0.08)*	
1	M	0.63	0.48	+
2	M	0.45	0.10	+
3	M	0.11	0.44	+
4	M	0.62	0.61	+
5	M	0.71	0.80	+
6	M	0.27	0.10	−
7	M	1.26	0.65	+
8	A	0.01	0.42	−
9	G	0	0.21	−

* Numbers within brackets denote mean values +3 SD from a control material of 60 subjects with tension headache.

Anti-MAG antibody secreting cells

Four of the 7 patients with polyneuropathy associated with IgM M component (Cases 1, 4, 6, 7; Table 3) had CSF cells producing anti-MAG IgM antibodies, in numbers varying between 1 per 212 and 1 per 3333 mononuclear cells in CSF. This finding indicates that these 4 patients had an ongoing intrathecal anti-MAG IgM antibody response. These 4 patients were all positive in CSF for anti-MAG IgM antibodies in ELISA, but the ELISA data do not support intrathecal antibody synthesis (Table 2) even though determinations were performed on standardized IgM amounts.

Surprisingly, the patient with polyneuropathy associated with an M component of IgA isotype (Case 8; Table 3) had in CSF a small number (1 per 10^4 CSF-L) of anti-MAG IgA antibody secreting cells, and the patient with polyneuropathy associated with an M component of IgG isotype (Case 9) had in CSF a high number (1 per 263 CSF-L) of anti-MAG IgG antibody secreting cells. Because of the small numbers of CSF-L available, it was not possible to determine anti-MAG IgM antibody secreting cells in the CSF from these 2 patients. Since both were positive for anti-MAG IgM antibodies in CSF—while negative in corresponding serum—we conclude that these 2 patients displayed evidence for the intrathecal synthesis of anti-MAG antibodies of 2 different isotypes in parallel.

In bone marrow, all 7 patients with an IgM M component had cells secreting anti-MAG IgM antibody. Their numbers varied between 1 per 370 and 1 per 50 000 (median 1 per 2000) BML. In contrast to CSF, no cells secreting anti-MAG IgG, IgA or IgM were detectable in bone marrow from Cases 8 and 9.

In peripheral blood, only 2 of the 7 patients with polyneuropathy associated with an IgM M component had cells secreting anti-MAG IgM antibody, numbering 1 per 5000 PBL (Case 3; Table 3) and 1 per 10^5 PBL (Case 6).

IgG, IgA and IgM secreting cells

The number of IgM secreting cells in bone marrow was higher in the 7 patients with polyneuropathy associated with an IgM M component (median 1 per 357 BML) than

TABLE 3 NUMBERS OF CELLS PER 10^4 CELLS IN CSF, BLOOD AND BONE MARROW (BM) SECRETING ANTI-MAG ANTIBODIES OF IgG, IgA AND IgM ISOTYPES, AND CORRESPONDING NUMBERS OF CELLS SECRETING IgG, IgA AND IgM (IN BRACKETS) IN 9 PATIENTS WITH POLYNEUROPATHY AND SERUM M COMPONENT

Case	*Isotype of serum M component*	*IgG*			*IgA*			*IgM*		
		CSF	*Blood*	*BM*	*CSF*	*Blood*	*BM*	*CSF*	*Blood*	*BM*
1	Mκ	nd (nd)	0.2 (2)	0 (6)	nd (nd)	0 (3)	0 (6)	4 (nd)	0 (2)	27 (57)
2	Mκ	nd (nd)	0 (3)	0 (9)	nd (nd)	0 (4)	0 (10)	0 (0)	0 (1)	8 (28)
3	Mλ	nd (3)	0 (6)	0 (9)	nd (nd)	0 (40)	0 (13)	0 (nd)	2 (8)	7 (28)
4	Mκ	nd (51)	nd (2)	nd (7)	nd (nd)	nd (1)	nd (8)	32 (nd)	0 (0.4)	0.2 (4)
5	Mκ	nd (36)	nd (1)	0 (10)	nd (nd)	nd (1)	nd (7)	0 (nd)	0 (0.4)	5 (17)
6	Mκ	nd (nd)	nd (9)	0 (6)	nd (nd)	nd (2)	nd (6)	47 (nd)	0.1 (2)	2 (22)
7	Mκ	nd (nd)	0 (10)	0 (12)	nd (nd)	nd (1)	nd (2)	3 (nd)	0 (3)	1 (48)
8	Aκ	nd (nd)	0 (2)	0 (12)	1 (6)	0 (5)	0 (125)	nd (nd)	0 (nd)	nd (nd)
9	Gλ	38 (nd)	0 (2)	0 (120)	nd (nd)	0 (1)	0 (18)	nd (nd)	0 (0.3)	0 (2)

nd = not done

TABLE 4. SUMMARY OF DATA FOR NUMBERS OF CELLS PER 10^4 CELLS IN CSF, BLOOD AND BONE MARROW (BM) SECRETING ANTI-MAG ANTIBODIES AND CORRESPONDING NUMBERS OF CELLS SECRETING Ig OF IgG, IgA AND IgM ISOTYPES IN 7 PATIENTS WITH POLYNEUROPATHY AND AN IgM M COMPONENT, AND OF IgG, IgA AND IgM SECRETING CELLS IN NORMAL CONTROLS

		IgG		*IgA*		*IgM*		
		Blood	*BM*	*Blood*	*BM*	*CSF*	*Blood*	*BM*
Anti-MAG antibody producing cells in polyneuropathy and IgM M component	Range	0–0.2	0	0	0	0–47	0–2	0.2–27
	Median (SD)					12 (18)	0.3 (0.7)	7 (9)
	No. pos/no. exam.	1/4	0/6	0/3	0/3	4/7	2/7	7/7
Ig producing cells in polyneuropathy and IgM M component	Range	1–10	6–12	1–40	2–13	0	0.4–8	4–57
	Mean (SD)	5 (4)	8 (2)	7 (14)	7 (3)		2 (3)	29 (18)
	No. pos/no. exam	7/7	7/7	7/7	7/7	0/1	7/7	7/7
Ig producing cells in controls	Range	1–9[1]	2–15	0–9[1]	2–15	nd	0–7[1]	1–4
	Mean (SD)	3 (2)	8 (5)	3 (3)	7 (4)	nd	2 (3)	2 (1)
	No. pos/no. exam.	25/25	7/7	23/25	7/7	nd	16/25	7/7

[1] From Olsson *et al.* (1990); nd = not done

in our 7 healthy controls (median 1 per 5000 BML) ($P < 0.02$; Table 4). No differences were seen in these patients versus controls for IgG or IgA secreting cells in bone marrow. Case 8 with an IgA M component had high numbers of IgA secreting cells in bone marrow (1 per 80 BML) and Case 9 with an IgG M component had high numbers of IgG secreting cells (1 per 83 BML).

In peripheral blood, the 7 patients with polyneuropathy associated with an IgM M component had IgM secreting cells at numbers similar to those found in 25 control subjects with tension headache (Table 4). Similarly, Cases 8 and 9 with M components of IgA and IgG, respecively, had no increase of IgA or IgG secreting cells in peripheral blood.

DISCUSSION

We have demonstrated anti-MAG IgM antibody secreting cells in CSF from patients with polyneuropathy associated with an M component of IgM isotype and anti-MAG IgM antibodies, thereby presenting direct evidence for the intrathecal synthesis of such antibodies in this disease. It is generally accepted that the presence of an M component in CSF in patients with a serum M component is the result of transudation from serum to CSF. This transudation is more prominent in those patients who have blood-brain barrier damage. However, our results from enumeration of anti-MAG antibody secreting cells suggest that synthesis of anti-MAG IgM antibodies occurs intrathecally in this disease. Hence, the presence of an M component in CSF could in part be due to local production.

In order to detect intrathecal antibody production we performed the anti-MAG ELISA with standardized CSF and serum IgM amounts. The 4 patients positive for cells secreting anti-MAG IgM antibody in CSF were negative in ELISA for anti-MAG IgM antibodies, while the reverse situation was observed in 2 other patients. The apparent discrepancy in the results obtained by the immunospot assay and ELISA can be explained by the different aspects of the B cell response that are measured: ELISA data reflect the level of specific antibodies after putative absorption and catabolism, while immunospot results reflect the occurrence of specific antibody secreting cells within the CSF at the time of lumbar puncture (Link, 1987, 1989). The two methods seem to be supplementary for detecting the intrathecal production of specific antibodies.

In the bone marrow, we detected anti-MAG IgM antibody secreting cells in all 7 patients who had a polyneuropathy associated with an IgM M component. These patients, as expected, also had high numbers of IgM secreting cells in their bone marrow. On the other hand, no anti-MAG antibody secreting cells were detectable in the 2 patients with polyneuropathy of IgA or IgG isotype, even though these patients had in their CSF cells secreting anti-MAG antibodies of corresponding isotype and, as expected, high numbers of IgA or IgG secreting cells in bone marrow.

The accumulation of cells secreting anti-MAG antibody and of immunoglobulin secreting cells in bone marrow and CSF in patients with polyneuropathy and an M component is not reflected in peripheral blood, where numbers of Ig secreting cells were in fact similar to those which we observed in healthy controls. The blood compartment thus gives a poor reflection of the synthesis of different immunoglobulins in the total body.

To our surprise, a patient with polyneuropathy associated with an IgA M component and a patient with polyneuropathy associated with IgG M component, had cells in CSF secreting anti-MAG antibodies of IgA and IgG isotype, respectively. Both patients were negative in CSF for anti-MAG antibodies by ELISA. Instead, they were both positive by ELISA for anti-MAG IgM antibodies, but in CSF only. We have thus obtained evidence for the concurrent synthesis within the CSF compartment of anti-MAG antibodies of 2 different isotypes in individual patients with polyneuropathy associated with M component. It is, however, not clear whether the same or different epitopes of MAG are recognized by the antibodies. The present technique makes it possible in future studies to clarify this question, and also define epitopes which are immunodominant.

The anti-MAG IgM antibodies that we detected in the 2 polyneuropathy patients with an M component of IgA or IgG isotypes do not belong to neoplastic plasma cell clones since anti-MAG antibody activity otherwise would have been detected in serum which contained high levels of the M component. Mechanisms for their induction remain unclear. One possibility is that they represent an immune response secondary to nerve damage.

Anti-MAG antibodies of IgM isotype when injected directly into peripheral nerves of suitable species induce demyelination (Hays *et al.*, 1987; Willison *et al.*, 1988). The production of anti-MAG antibodies which we have demonstrated within the CSF compartment might have importance for the development of polyneuropathy. These antibodies might reach their target in the peripheral nervous system, at least at nerve root level, without the need for passage from serum through the blood-nerve barrier. Direct anti-MAG antibody mediated damage may, on the other hand, not be the only mechanism involved in the pathogenesis of the polyneuropathy in these patients. Our recent observation of infiltrations of T cells in sural nerve biopsies from patients with polyneuropathy and monoclonal gammopathy of undetermined significance (MGUS) of IgM and IgG isotypes (G. Solders *et al.*, unpublished) might indicate that this cell type is also involved.

In conclusion, we have demonstrated the intrathecal production of anti-MAG IgM antibodies in patients with polyneuropathy and MGUS. In 2 of the patients we found evidence for the concurrent intrathecal synthesis of anti-MAG antibodies of two different isotypes. Our findings suggest that it could be of value to examine CSF in parallel with serum for demonstration of anti-MAG antibodies. Studies are warranted which focus on the possible importance of these locally produced antibodies in the pathogenesis of polyneuropathy in patients with MGUS.

ACKNOWLEDGEMENTS

This study was supported by the Swedish Medical Research Council (project nos 3381 and 7488) and by the Swedish MS Society (NHR). We thank Ms Yvonne Nilsson for excellent secretarial assistance.

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(Received December 21, 1989. Revised March 27, 1990. Accepted April 10, 1990)

Brain (1991), **114**, 585–599

MORPHOLOGICAL CHANGES IN UNMYELINATED NERVE FIBRES IN THE SURAL NERVE WITH AGE

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SUMMARY

Quantitative changes in unmyelinated nerve fibres in sural nerves obtained at autopsy were evaluated in 28 normal adults. The following conclusions were reached. (1) The density of unmyelinated axons showed no significant correlation with age, but the densities of (2) Schwann cell subunits with axons, (3) Schwann cell subunits without axons, (4) single protrusions of Schwann cells and (5) collagen pockets, and (6) the mean number of Schwann cell profiles per axon, all showed positive correlations with age. Additionally, (7) the percentage of subunits containing unmyelinated axons and (8) the mean number of axons in single axon-containing Schwann cell subunits demonstrated negative correlations with age. The density of Schwann cell nuclei related to unmyelinated fibres did not show a significant change with age. The age-dependent changes in unmyelinated nerve fibres thus mainly consist of an increased production of processes by Schwann cells in the absence of cell multiplication. A decrease in unmyelinated nerve fibre density or a compensatory increase of small unmyelinated axons did not occur in these normal adults. In terms of relative sensitivity for the detection of the earliest changes in unmyelinated fibres, the indices (6) and (7) are considered to be useful and superior to the conventional assessment of unmyelinated axon density and diameter distribution. These two indices are not influenced by postmortem swelling of the axons and Schwann cells. Measurements of unmyelinated axon density and size distribution will continue to be useful in the assessment of more advanced pathological conditions.

INTRODUCTION

Unmyelinated axons are one of the predominant constituents of cutaneous nerves. They are considered to represent postganglionic sympathetic efferents as well as sensory afferents subserving such modalities as pain and temperature. Although they are about three to five times as numerous as myelinated fibres, they have largely been neglected in routine neuropathological examinations, especially at autopsy. One reason for this is that electron microscopy is required because of their small size (0.1–3.0 μm diameter). Another more significant reason is that the postmortem changes, including axonal swelling, may invalidate morphometric findings concerning their density and the size distribution, both of which have been considered to be essential markers for the evaluation of the changes in unmyelinated nerve fibres.

However, as Behse *et al.* (1975) and Ochoa (1978) have reported, the earliest change in unmyelinated nerve fibres in chronic neuropathies is an increased number of cytoplasmic processes projecting from Schwann cells, followed by increased numbers of Schwann cell bands devoid of axons ('empty Schwann cell subunits'), with or without

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a diminution in the number of unmyelinated axons. Thus the morphometric assessment of the number of Schwann cell profiles per axon and the percentage of Schwann cell subunits containing unmyelinated axons may be more sensitive for the detection of early changes in unmyelinated nerve fibres than measurements of the density or the diameter histogram of the unmyelinated axons. Moreover, the former two indicators are little influenced by postmortem change. The aim of the present study is to provide basic data for the evaluation of the unmyelinated nerve fibres in the sural nerve in autopsied cases from different age groups, by a morphometric analysis predominantly employing these two indices, in comparison with the conventional axonal density estimations or diameter histograms. The densities of Schwann cell nuclei related to unmyelinated nerve fibres, Schwann cell subunits with or without axons, isolated projections from Schwann cells, collagen pockets (Gamble and Eames, 1964) and the mean number of unmyelinated axons in single axon-containing subunits are also evaluated.

MATERIAL AND METHODS

Sural nerves were obtained from 20 males and 8 females, aged 25–89 yrs, all within 18 h of death (Table 1); 18 samples were taken within 6 h, and 5 of the remaining 10 cases with delays in removal of over 6 h were stored in refrigerators (4° C) immediately after death. Two-thirds of the subjects had been examined by 2 or more neurologists and had been diagnosed as not suffering from peripheral neuropathy.

TABLE 1. CAUSE OF DEATH AND DELAY IN REMOVAL OF SURAL NERVES

Case	*Age (yrs)/sex*	*Cause of death*	*Delay in removal of sural nerves (h)*
1	25 F	Suicide	4
2	34 M	Acute leukemia	2
3	40 F	Sudden death	6
4	43 F	Pneumonia	14
5	43 F	Ruptured berry aneurysm	5
6	46 M	Glioma	15
7	47 M	Cardiomyopathy (fatal arrhythmia)	2
8	48 F	Carcinoma of ovary	3
9	52 M	Ruptured berry aneurysm	11
10	56 M	Cerebral infarct	3
11	58 M	Carcinoma of oesophagus	2
12	62 M	Cerebral infarct	18
13	65 M	Malignant lymphoma of brain	3
14	66 M	Hepatoma	6
15	66 M	Cerebral haemorrhage	2
16	67 M	Carcinoma of lung	3
17	68 M	Carcinoma of lung	3
18	69 M	Cerebral infarct	12
19	70 F	Cerebral haemorrhage	4
20	71 M	Cerebral infarct	9
21	73 M	Carcinoma of lung	3
22	73 M	Carcinoma of lung	3
23	75 M	Cerebral infarct	2
24	80 M	Cerebral infarct	13
25	81 M	Cerebral infarct	15
26	85 F	Cerebral infarct	13
27	88 F	Ruptured berry aneurysm	2
28	89 M	Cerebral infarct	2

Patients with diabetes mellitus, chronic renal failure, systemic vasculitis or paraproteinaemia were not included in the study.

Specimens were taken just proximal to the lateral malleolus. A 3 cm length of nerve was removed, fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer, postfixed in 1% osmium tetroxide and embedded in Epon. Ultrathin sections were stained with uranyl acetate and lead citrate and then observed in a JEOL 200CX electron microscope.

Morphometry

Ultrathin sections were obtained from at least 3 fascicles at the same plane, and the total area analysed in electron micrographs from each nerve was at least 0.03 mm^2. The final magnification used for measurement was ×10 000. Because the fascicles were uneven, the ultrathin sections were not always cut at right angles to the long axis of the sural nerve specimen; hence, in some subunits, the boundary of each cytoplasmic process of Schwann cell was not clear. We marked these subunits on the electron micrographs and reexamined them in the electron microscope to count the number of their cytoplasmic processes, at a magnification of ×25 000, with the aid of a tilting stage to clarify the demarcation of the adjacent cell processes.

Unmyelinated axons were distinguished from Schwann cell profiles by their round or oval shape and the presence of a mesaxon-like structure. A higher incidence of microtubules or a lighter appearance than Schwann cell cytoplasm, characteristic appearances of the unmyelinated axons observed in biopsy material, cannot be used as morphological criteria because the microtubules in the axon sometimes diminish in number and the cytoplasm of Schwann cells often becomes as light as that of unmyelinated axons because of postmortem changes, especially in the cases without preservation in the refrigerator (fig. 3). With the criteria stated above, counts made by two observers gave identical results within 5%.

In this study a conglomerate of Schwann cell processes with or without unmyelinated axons, which was enclosed by a continuous basal lamina, was designated as a Schwann cell 'subunit' (Sharma and Thomas, 1975). The isolated projections of Schwann cells made up of a single profile unassociated with an axon on cross-section, considered to be derived from an outgrowth from or shrinkage of Schwann cells, were counted separately, because these structures did not persist longitudinally—they ended blindly in the endoneurium or appeared as an additional profile comprising another subunit at a distance of 5 – 10 μm when observed in serial sections (Behse *et al.*, 1975). The percentage of Schwann cell subunits containing unmyelinated axons and the mean number of Schwann cell profiles per axon in the unmyelinated axon-containing Schwann cell subunits were counted in each case and were evaluated in relation to age. The densities of Schwann cell nuclei related to unmyelinated nerve fibres, Schwann cell subunits with or without unmyelinated axons, isolated projections of Schwann cells, and collagen pockets (Gamble and Eames, 1964), in addition to the conventional unmyelinated axon density, were also estimated. For their quantitative evaluation, we defined 'collagen pockets' as small collections of longitudinally oriented collagen fibrils, round or oval in shape, surrounded by Schwann cell processes over at least three-quarters of their perimeter.

Bands of Büngner, i.e., Schwann cells which were previously related to myelinated axons, were not included in these counts. Unmyelinated axons which occurred in them also were not counted. Bands of Büngner were distinguished by the following morphological criteria (Ochoa and Mair, 1969*b*; Behse *et al.*, 1975) from subunits of Schwann cells associated with unmyelinated axons: (1) they have a larger diameter (3 – 8 μm) than subunits associated with unmyelinated axons; (2) each of their profiles is bigger than that in the subunits associated with unmyelinated axons; (3) they may contain remnants of myelin and lamellated inclusions; and (4) their shape is more irregular, and the basement membrane folded. Unmyelinated axons that occurred in onion-bulb formations, which were tiny and usually did not exceed one lamina in these normal subjects, also were not included in the counts of unmyelinated axons because it could not be decided whether they were derived from regenerating myelinated fibres or cross-innervation from unmyelinated axons. Similarly, Schwann cell subunits which participated in the onion-bulb formations also were not included because it could not be decided whether they were originally associated with myelinated or unmyelinated axons, as pointed out by Behse *et al.* (1975). Unmyelinated axons and subunits which took part in the formation of regenerating 'clusters' of myelinated nerve fibres also were not counted for the same reason.

Measurements of the diameter of unmyelinated axons and statistical analysis were performed using a WT-4400 digitizer (WACOM K.K.) interfaced to a Nihon Denki PC9801VM personal computer.

RESULTS

Qualitative observations

In younger cases, almost all the Schwann cell subunits contained axons with well-preserved mesaxons and empty Schwann cell subunits were rarely found. On the other hand, the number of Schwann cell profiles within one Schwann cell subunit was greater and empty subunits were frequently observed in the elderly (figs 1, 2). Small onion-bulb formations and clusters of small regenerating myelinated nerve fibres, having some Schwann cell subunits around them, also increased in the older age groups. The collagen pockets were not evenly distributed in the transverse plane and were observed to be clustered in some regions.

Postmortem changes

It has generally been considered that unmyelinated nerve fibres readily undergo marked postmortem changes; this is another reason which has led to their neglect at autopsy. The present study therefore needs to describe the details of the postmortem changes in these specimens and to justify the use of sural nerves with a maximum delay in removal of 18 h.

All the material examined showed well-preserved axonal and Schwann cell surface membranes, and Schwann cell basement membranes; they were adequate for ultrastructural analysis. Representative photographs of cases with early and delayed (with and without refrigeration) removal are shown in fig. 3. In cases with early removal (within 6 h of death), some cytoplasmic structures including mitochondria became swollen but the axonal organelles (e.g., microtubules) were well preserved and the relatively lighter appearance of the axons as compared with Schwann cell processes (Dyck and Lambert, 1969) was clearly evident.

Significant postmortem changes were observed, most usually in those cases with a delay in removal of 8 – 10 h. In some cases unmyelinated axons were severely expanded and their axonal organelles had disappeared. Schwann cells around the unmyelinated axons became swollen and their processes and the unmyelinated axons were almost equally electron-dense, and occasionally the latter were rather darker than the former. However, in such cases, the relationship between the axons and Schwann cell processes was retained and the number of Schwann cell profiles in one subunit was never influenced by postmortem changes. In some cases which had been stored in refrigerators, the axonal organelles were well preserved and the axons themselves were not as expanded (fig. 3B).

Morphometric results

Quantitative data from the 28 cases are shown in Table 2.

Density and diameter distribution of unmyelinated axons. The density of unmyelinated axons in the 28 cases ranged between 19 040 and 42 520/mm^2, as shown in Table 2 and fig. 3. The diameter histogram for all of them showed a unimodal distribution, with the peak at 0.5 – 1.4 μm. No correlation could be found between the density of unmyelinated axons and the patient's age (fig. 4A). The mean diameter of the unmyelinated axons in the 5 cases with delays in removal of the sural nerves over 6 h and without use of refrigeration was significantly larger than that in the cases with delays of less than 6 h (fig. 5).

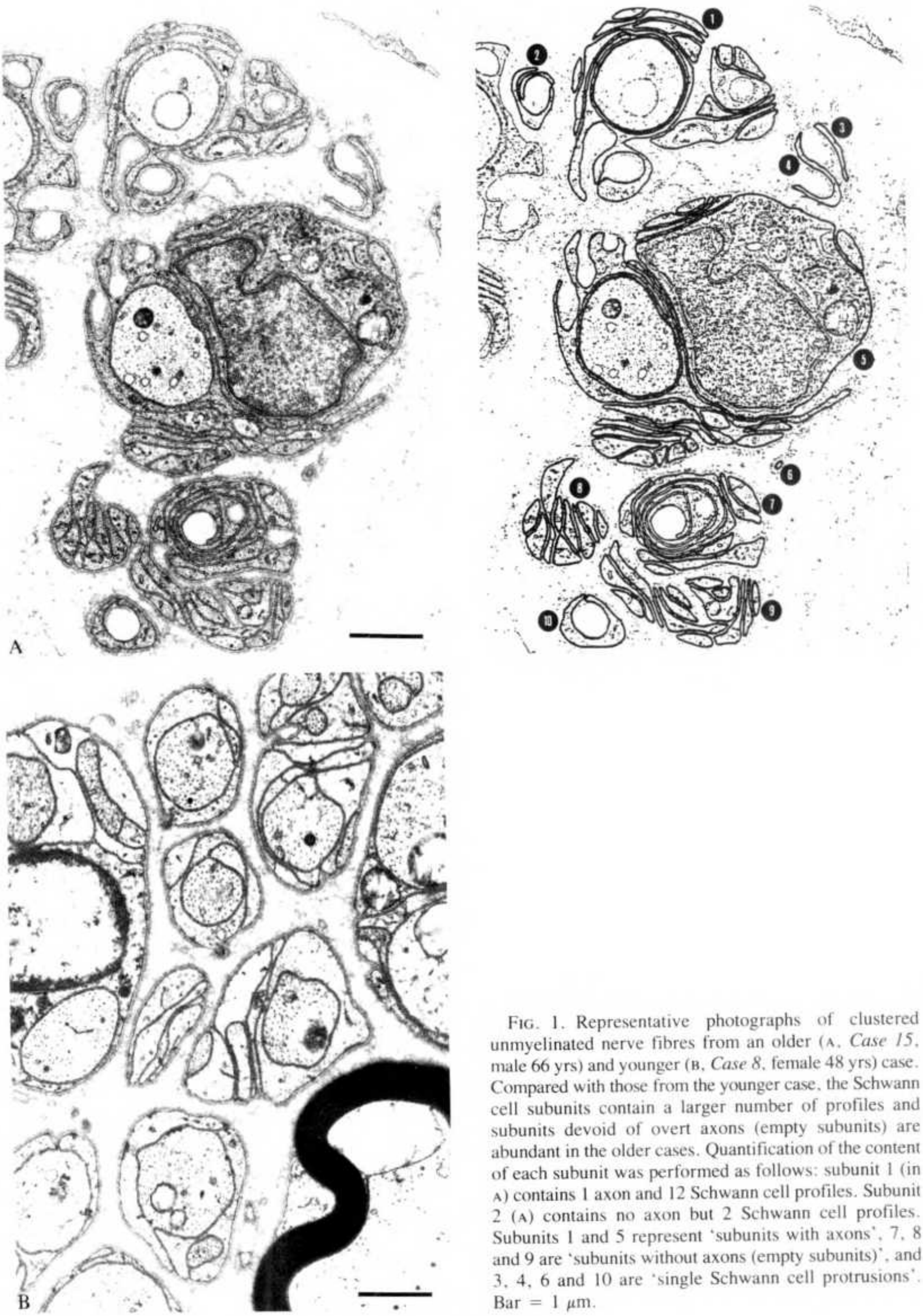

FIG. 1. Representative photographs of clustered unmyelinated nerve fibres from an older (A, *Case 15*, male 66 yrs) and younger (B, *Case 8*, female 48 yrs) case. Compared with those from the younger case, the Schwann cell subunits contain a larger number of profiles and subunits devoid of overt axons (empty subunits) are abundant in the older cases. Quantification of the content of each subunit was performed as follows: subunit 1 (in A) contains 1 axon and 12 Schwann cell profiles. Subunit 2 (A) contains no axon but 2 Schwann cell profiles. Subunits 1 and 5 represent 'subunits with axons', 7, 8 and 9 are 'subunits without axons (empty subunits)', and 3, 4, 6 and 10 are 'single Schwann cell protrusions'. Bar = 1 μm.

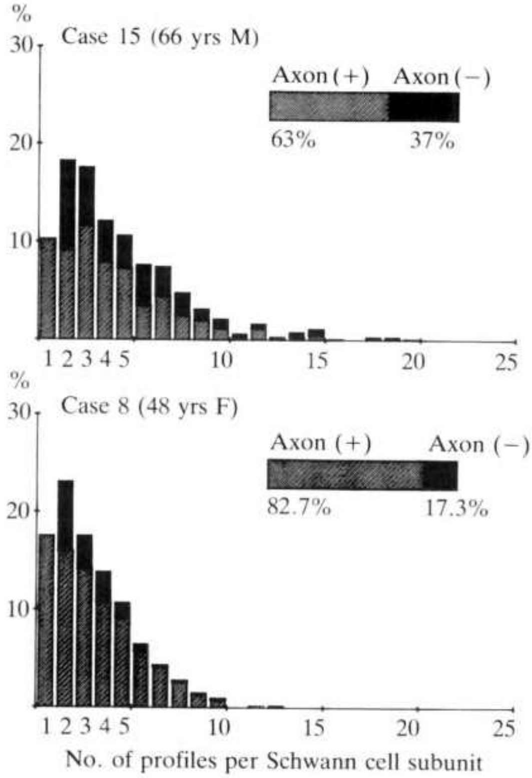

FIG. 2. Representative percentage frequency histograms of the number of profiles per Schwann cell subunit in the older and younger cases as described in fig. 1. Hatched bars show the number of Schwann cell profiles in axon-containing subunits, and black bars the number in subunits devoid of axons (empty subunits). Single protrusions are not included in this histogram. Subunits from older cases contain many more profiles and have a higher percentage of subunits devoid of axons.

Density of Schwann cell nuclei of unmyelinated nerve fibres. This index ranged from 700 to 3900/mm^2 in these 28 cases and showed no significant correlation with age (fig. 4B).

Densities of Schwann cell subunits with and without axons, single Schwann cell protrusions and collagen pockets. The values for these 4 parameters were 11 670−31 640, 700−16 280, 2410−50 180 and 2340−18 970/mm^2, respectively. A weak but significant correlation ($P < 0.05$) was found between the density of subunits with axons and the age of the subject (fig. 6A). A higher correlation ($P < 0.01$) existed between the density of Schwann cell subunits without axons (fig. 6B), and between the density of single Schwann cell protrusion and the subject's age (fig. 6C). The density of collagen pockets showed a tendency to increase with age: the value in younger controls (under 50 yrs) did not exceed 10 000/mm^2, but the values were scattered in older controls (fig. 6D). As a whole, a weak ($P < 0.05$) correlation could be found with age.

Percentage of subunits containing unmyelinated axons. This index ranged from 55.7 to 94.3% in these 28 patients. All 9 cases aged over 70 yrs (Cases 20−28) showed

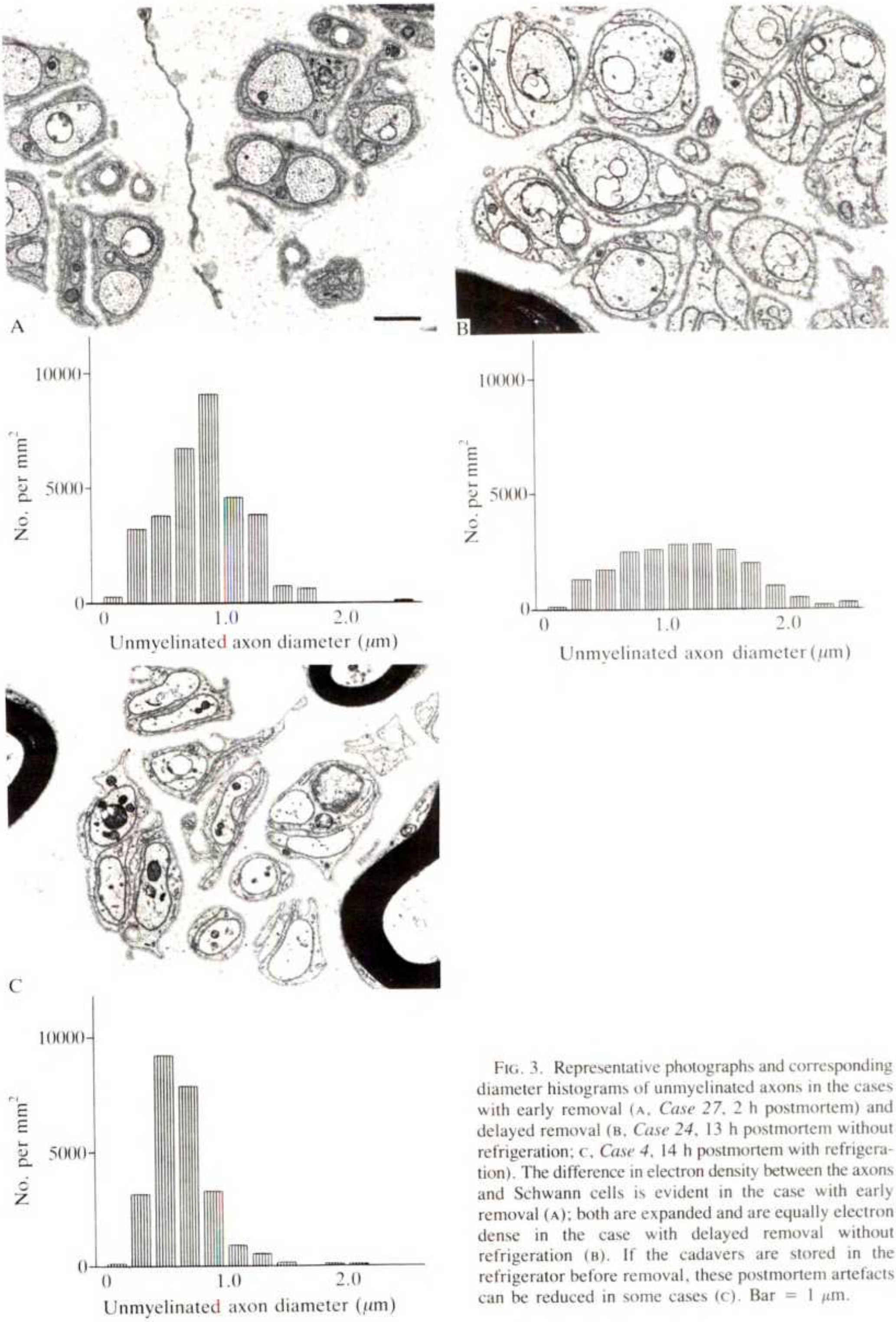

FIG. 3. Representative photographs and corresponding diameter histograms of unmyelinated axons in the cases with early removal (A, *Case 27*, 2 h postmortem) and delayed removal (B, *Case 24*, 13 h postmortem without refrigeration; C, *Case 4*, 14 h postmortem with refrigeration). The difference in electron density between the axons and Schwann cells is evident in the case with early removal (A); both are expanded and are equally electron dense in the case with delayed removal without refrigeration (B). If the cadavers are stored in the refrigerator before removal, these postmortem artefacts can be reduced in some cases (C). Bar = 1 μm.

TABLE 2. MORPHOMETRIC RESULTS

		Densities/mm^2 of							
Case	*Unmyelinated axons*	*Schwann cell nuclei**	*Subunits with axons*	*Subunits without axons*	*Single protrusions*	*Collagen pockets*	*Percentage of subunits containing axons*	*Mean no of profiles per axon*	*Mean no of axons in single subunits*
1	20 220	700	11 670	700	2410	2340	94.3	1.49	1.71
2	27 080	2410	17 840	4410	15 850	6090	80.2	2.49	1.52
3	35 060	2150	16 300	1900	9230	5800	89.6	1.62	2.15
4	25 050	1380	14 890	2450	10 820	5200	85.9	1.67	1.68
5	29 280	2390	21 660	5620	14 840	6020	79.4	2.94	1.35
6	23 230	840	17 210	5020	12 930	6180	77.4	3.02	1.35
7	22 120	1990	14 050	2390	10 910	3490	85.5	2.81	1.52
8	25 000	1260	17 190	3600	11 030	3420	82.7	2.59	1.45
9	42 520	3290	31 640	13 920	18 480	14 430	69.4	2.72	1.35
10	21 760	1550	17 820	3170	15 630	3450	84.9	3.04	1.22
11	24 300	1300	18 960	4540	12 400	4690	80.7	2.49	1.28
12	30 040	2510	22 040	7200	24 210	3660	75.4	2.87	1.36
13	29 900	3170	24 150	15 540	20 290	7130	60.8	4.00	1.26
14	35 970	1810	27 710	5940	17 930	9070	82.3	2.56	1.30
15	28 900	1880	25 410	14 950	23 070	13 210	63.0	3.91	1.18
16	24 840	1760	18 150	5020	14 360	5200	78.3	2.77	1.37
17	30 260	1960	21 760	5160	21 640	11 560	80.8	1.73	1.39
18	21 320	1330	18 430	14 210	24 650	3180	56.5	3.79	1.16
19	33 950	2180	24 810	7800	25 290	6140	76.1	3.15	1.37
20	24 350	1670	20 930	9130	36 140	11 370	69.6	2.67	1.18
21	30 090	2240	24 070	10 620	22 750	17 420	69.4	2.72	1.25
22	19 040	940	15 120	7270	11 770	7050	67.5	4.28	1.26
23	22 880	1710	16 040	7380	20 400	3890	68.5	4.70	1.43
24	20 000	1880	19 230	15 300	34 620	5560	55.7	3.56	1.04
25	24 960	2080	22 740	13 070	10 710	16 360	63.5	3.23	1.20
26	33 230	3900	29 730	16 280	50 180	18 970	64.6	2.52	1.12
27	32 710	2210	23 330	12 580	31 340	8620	65.0	3.01	1.38
28	25 000	2250	18 660	11 460	11 560	3890	62.0	3.17	1.19

* Only those related to unmyelinated fibres are counted.

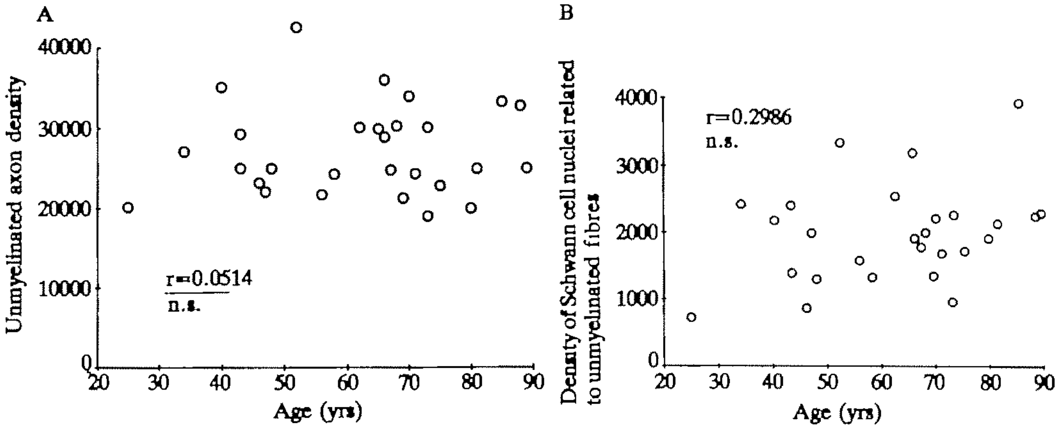

FIG. 4 Relationship between (A) unmyelinated nerve fibre density and (B) density of Schwann cell nuclei associated with unmyelinated fibres, and age. No significant correlation is evident for either parameter

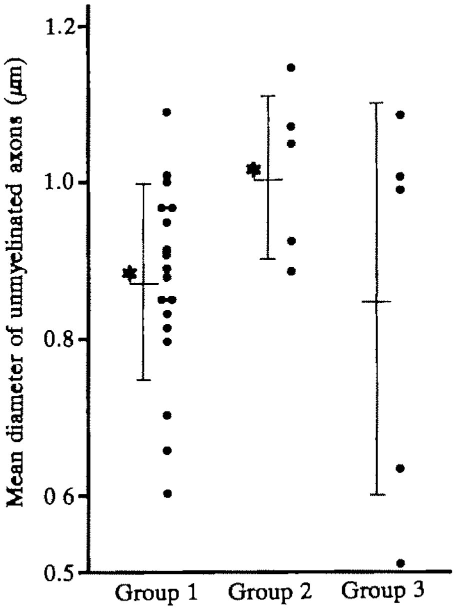

FIG. 5. Scattergram of the diameter of unmyelinated axons in the three groups. Group 1 includes the cases with early removal (6 h postmortem or less) and groups 2 and 3 cases with delayed removal (group 2. without refrigeration; group 3: with refrigeration). The mean diameter of axons in group 2 is significantly ($P < 0.05$, asterisk) larger than that in group 1 The values in group 3 are greatly scattered and the postmortem expansion of the axons was not reduced in 3 out of the 5 cases. Bar = mean ±SD

values below 70%. There was a good negative correlation between the percentage of subunits containing unmyelinated axons and the subject's age (fig. 7A).

Mean number of Schwann cell profiles per axon. The mean number of Schwann cell profiles per axon in the axon-containing Schwann cell subunits ranged between 1.49 and 4.70, and increased significantly with age (fig. 7B).

Mean number of axons in axon-containing Schwann cell subunits. This ranged between 1.04 and 2.15 and decreased significantly with age (fig. 7C).

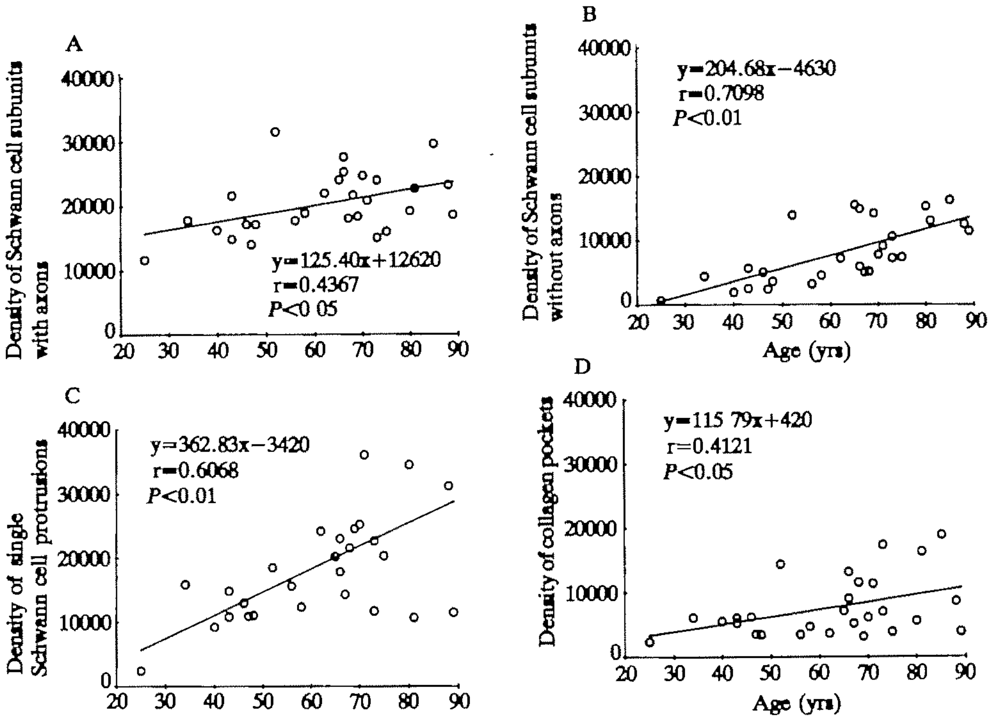

FIG 6. Relationship between the density of (A) Schwann cell subunits with axons, (B) Schwann cell subunits without axons, (C) single Schwann cell protrusions, (D) collagen pockets, and age. *See* text for explanation.

DISCUSSION

Quantitative analysis of the unmyelinated nerve fibres in peripheral nerve has been performed in a number of neurological disorders, such as alcoholic neuropathy (Behse and Buchthal, 1977), acute pandysautonomia (Low *et al.*, 1983), diabetic neuropathy (Kanda, 1984), xeroderma pigmentosum (Kanda *et al.*, 1990). In most of these studies, a diminution in the density of axons and changes in their size distribution have been the main consideration in evaluating the morphological changes in unmyelinated nerve fibres. However, these two parameters are not particularly sensitive and the reported 'normal' range for the density of unmyelinated axons has been found to be very wide (Ochoa and Mair, 1969*a*; Ochoa, 1978). Relatively subtle or early changes in unmyelinated nerve fibres which did not influence their density would be overlooked if assessment was limited to the measurement of density. In some reports (Dyck and Lambert, 1969; Aguayo *et al.*, 1971; Ohta *et al.*, 1973; Thomas and King, 1974; Kanda *et al.*, 1989), increased numbers of denervated Schwann cell subunits or of the Schwann cell profiles within the subunits were also described, although not quantitatively. Behse and Buchthal (1977) assessed unmyelinated nerve fibre density and Schwann cell subunits containing or devoid of unmyelinated axons in 13 cases of alcoholic neuropathy. They found a decrease in the density of unmyelinated axons in 6 cases and, in 4 cases, numerous empty Schwann cell subunits not accompanied by a reduction of unmyelinated axons.

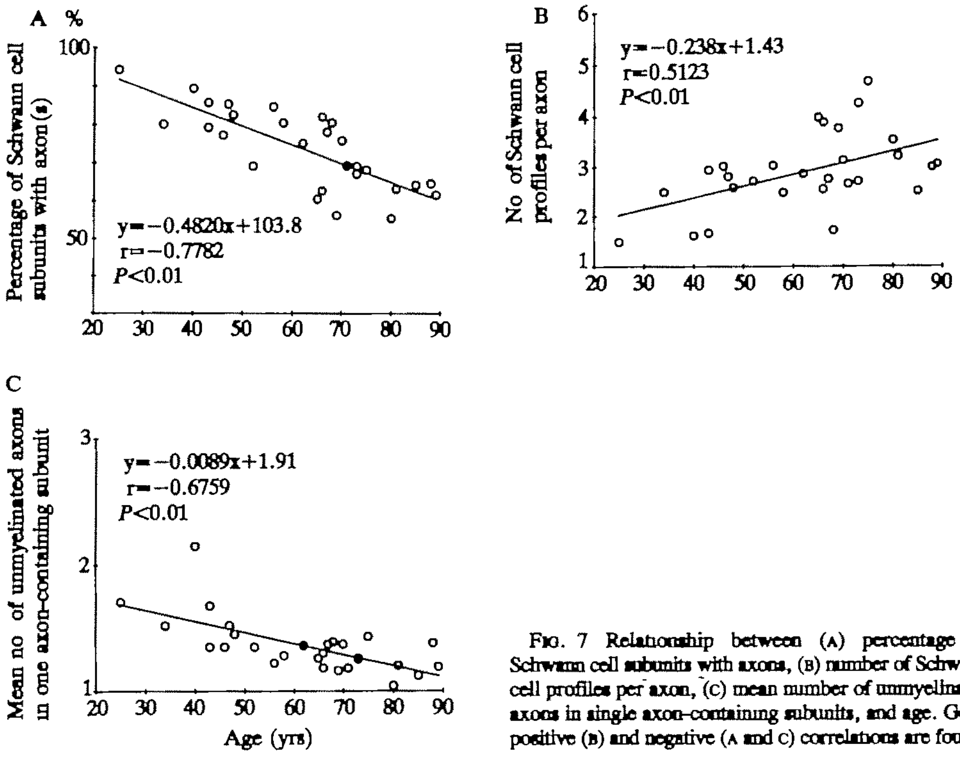

FIG. 7 Relationship between (A) percentage of Schwann cell subunits with axons, (B) number of Schwann cell profiles per axon, (C) mean number of unmyelinated axons in single axon-containing subunits, and age. Good positive (B) and negative (A and C) correlations are found.

Their results suggested that morphometric analyses directed towards changes in the Schwann cells of unmyelinated nerve fibres would be more sensitive than conventional density estimations, and that for the precise evaluation of the changes in unmyelinated nerve fibres, including alterations in the Schwann cells, control values for these changes in various age groups must be established.

Quantitative data for normal subjects are still rare; if the material for investigation is limited to biopsied sural nerves, the controls are required to be obtained from healthy volunteers and so the majority of the articles have used and discussed no more than 5–7 normal controls. In this study, we used autopsy material to establish control values for unmyelinated nerve fibres. One of the merits of this is that material from a large number of subjects can be obtained to establish the characteristic morphological features in various age groups. But the limitations must also be taken into consideration. Jacobs and Love (1985) studied 27 sural nerves from 'normal' subjects obtained at autopsy. This has been the largest series concerning the changes in unmyelinated nerve fibres with age. They described a consistent reduction in the density of unmyelinated axons and a relative increase of small unmyelinated axons in the elderly. Ochoa and Mair (1969*b*) also reported bimodality in the unmyelinated axon size-frequency distributions in older subjects using biopsied sural nerves. We failed to find any correlation between the density of unmyelinated axons and ageing, or a relative increase of small unmyelinated axons in aged subjects. A failure to detect bimodality in the unmyelinated axon size-frequency distributions in older subjects was also described by Sharma and Thomas

(1975), in a study using the abdominal vagus nerve. The discrepancies between our data and those of Jacobs and Love concerning the density of unmyelinated axons may be partly explained by the difference in the subject's ages: in their paper, a marked decrease in the density of unmyelinated axons with age occurred mainly during the early years of life and the age-dependent reduction was not as apparent in the adult. An increase in small axons in the elderly (Ochoa and Mair, 1969*b*; Jacobs and Love, 1985) may partly be derived from differences in the criteria used to distinguish the tiny axons from the small Schwann cell processes.

We found a significant relationship between the mode of the frequency histogram of unmyelinated axon diameter and the time after death. The mean diameter of unmyelinated axons in the cases with delayed removal without use of refrigeration was significantly larger than in the cases with early removal, a reflection of postmortem swelling of the unmyelinated axons. This indicates that autopsy material is not adequate if conventional diameter histograms are used as an index of the changes of unmyelinated nerve fibres. Jacobs and Love (1985) mentioned that well-preserved ultrastructure of the autopsied sural nerves may be explained by their relatively distal and superficial location, probably resulting in rapid cooling afer death. Institution of refrigeration immediately after death enhanced this advantage in some cases, but we concluded that the pattern of the unmyelinated axon size-frequency distributions should not be used as a parameter for changes in unmyelinated nerve fibres of autopsied cases, because postmortem swelling of axons and Schwann cells is inevitable and because the degree of amelioration of postmortem artefacts by use of refrigerators is different in each case.

There is little previous information on the changes in the Schwann cells of unmyelinated fibres with respect to ageing processes. Our morphometric investigations used the indices of (1) the mean number of Schwann cell profiles per unmyelinated axon in a Schwann cell subunit, and (2) the percentage of Schwann cell subunits containing unmyelinated axons. We found a positive correlation with age for the former and a negative correlation for the latter; both were significant. Increases in the number of Schwann cell profiles in a Schwann cell subunit with age has been mentioned in some reports (Ochoa and Mair, 1969*b*; Schröder and Gibbels, 1977), although no quantitative analysis in association with ageing has yet been performed. Compared with the number of Schwann cell profiles, Schwann cell subunits without unmyelinated axons or denervated Schwann cell bands appear to have been examined more closely by some previous authors. Ochoa and Mair (1969*b*) reported that their densities were about 1245–3700/mm^2 in men aged 34 and 59 yrs, respectively, while they were less than 1000/mm^2 in individuals aged 32 and under. Schröder and Gibbels (1977) studied 2 senile control subjects (aged 83 and 88 yrs) and stated that the empty Schwann cells might be related to ageing or other cause of slow axonal wastage with Schwann cell proliferation. Our results support their view that Schwann cell subunits without unmyelinated axons increase with age.

The origin of the Schwann cell subunits without unmyelinated axons is still in dispute. Some authors thought of them as the residue of axonal loss (Ochoa and Mair, 1969*b*; Ochoa, 1970); others considered that they were pathological new additions of collateral Schwann cell appendages (Behse *et al.*, 1975). In the present study, increases of subunits devoid of axons with age did not occur concomitantly with a decrease in unmyelinated nerve fibre density but accompanied an increase of the number of Schwann cell profiles in a subunit and in the density of isolated Schwann cell projections. On the other hand, the density of Schwann cell nuclei related to unmyelinated nerve fibres did not increase significantly with age. These quantitative data suggest that these structures are derived from an enhanced proliferative activity of Schwann cells without multiplication, rather than from the simple loss of their axons. However, the normal range for the density of unmyelinated axons in the adult was as wide as 19 000–65 000/mm^2 in previous reported studies (Ochoa and Mair, 1969*a*; Behse *et al.*, 1975; Ochoa, 1978). It was 19 040–42 520/mm^2 in this study. A substantial loss of axons might still leave the density within the normal range. In our data the mean number of axons in single axon-containing Schwann cell subunits decreased significantly with age. Probably this does not mean that a loss of unmyelinated axons occurred in the older age groups but that an increase in the number of axons in an individual subunit due to regeneration did not occur even in older controls, although the former possibility cannot be negated. It is thus not possible to deny completely the hypothesis that the subunits without unmyelinated axons reflect axonal loss.

Another important problem regarding the origin of empty subunits should be mentioned. In the present study, we did not count the subunits forming a part of regenerating 'clusters' of myelinated nerve fibres or the ones taking part in the formation of an onion-bulb change. Larger subunits which were considered to be Schwann cells derived from myelinated nerve fibres (Büngner bands), sometimes surrounded by redundant basement membranes or with pi granules in their cytoplasm, were also excluded from the counts. Although some admixture of Schwann cell subunits of previously myelinated nerve fibres is not completely avoidable, we believe that their percentage is negligible in the present study.

The origin of collagen pockets is also still in dispute. Some authors have considered them to be another indicator of impairment of unmyelinated nerve fibres. Thomas (1973) thought that they mark the original location of unmyelinated axons which have degenerated and disappeared. However, in their three-dimensional reconstructions of unmyelinated fibres, Carlsen *et al.* (1974) reported that they were usually related to short isolated Schwann cell processes located externally within the Schwann cell subunits. Gamble (1964) regarded them as the consequence of active engulfing by Schwann cells of suitably orientated elongated structures. We found a weak but significant correlation between the density of collagen pockets and that of the Schwann cell subunits without axons; this suggests that the formation of collagen pockets is related to the proliferative activity of Schwann cells and does not mean the simple loss of unmyelinated axons.

We found a weak but significant correlation between the density of collagen pockets and the subject's age. Ochoa (1971) found no collagen pockets in fetal nerve before 18 weeks, and many authors have considered that they are related to ageing (Thomas and Ochoa, 1984). However, there has been no previous quantitative study concerning the relationship between their density and age in the normal human sural nerve. In our study, collagen pockets were not evenly distributed and were found to be concentrated in some areas in transverse sections of the nerve. In younger individuals (under 50 yrs), the density of collagen pockets did not exceed 10 000/mm^2; higher values were observed in some of the older controls (over 50 yrs), although the values were scattered in these cases. Consequently, we conclude that this index is not superior to the other indices described in this paper; for example, the density of Schwann cell subunits without axons or the percentage of Schwann cell subunits with axons, as a sign of ageing. The use of the frequency of collagen pockets as a reliable index of ageing needs reconsideration.

Finally, a further advantage in the use of the indices of (1) the mean number of Schwann cell profiles per unmyelinated axon in a Schwann cell subunit and (2) the percentage of Schwann cell subunits containing unmyelinated axons for the evaluation of the changes of unmyelinated nerve fibres in comparison with the conventional morphometric methods should be pointed out. To count the density of unmyelinated axons and obtain a histogram for axon diameter, randomly taken electron micrographs which cover an area of at least 0.02 mm^2 are required. As the unmyelinated nerve fibres are not evenly distributed but have some tendency to be clustered in certain regions and to form the groups consisting of 10–40 axons in the transverse plane, two-thirds or more of the photographs were usually occupied by myelinated nerve fibres. Determination of the density of Schwann cell subunits devoid of axons (empty subunits), a more sensitive indicator to evaluate the early changes of unmyelinated axons and their Schwann cells, has the same problems. On the other hand, to estimate the two indices described above, calculation of densities is not required and the photographs can be taken only in the area of the groups of the unmyelinated nerve fibres: it saves the cost of photographs and labour. Although there is a need to know the density of unmyelinated axons for the complete evaluation of the changes in unmyelinated fibres, particularly in more advanced pathological conditions, we suggest that these two indices should be used more widely in the future because of their sensitivity and time-saving properties, especially in autopsied cases.

ACKNOWLEDGEMENTS

The authors wish to thank Drs Fumiko Tamada and Masayuki Ikeda for providing specimens, and Ms Koko Ishii and Messrs Mitsuru Takamuku and Kenshiro Kobayasi for their excellent technical assistance.

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(*Received December 28, 1989. Revised March 16, 1990. Accepted April 10, 1990*)

Brain (1991), **114**, 601–614

SUBCUTANEOUS LISURIDE INFUSION IN PARKINSON'S DISEASE

RESPONSE TO CHRONIC ADMINISTRATION IN 34 PATIENTS

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SUMMARY

Thirty-eight parkinsonian patients with motor fluctuations and dyskinesias on chronic levodopa therapy were treated with subcutaneous lisuride infusion (SLI). Thirty-six were discharged from hospital on combined treatment with 24 h lisuride infusion (111.3 ± 29.5 μg/h) and oral levodopa plus a decarboxylase inhibitor (729.6 ± 452 mg/day); all obtained a marked initial improvement in mobility (mean reduction 'off' hours 87.5%); 34 were followed for a mean of 20.85 (range 6–45) months. Most patients continued to show improvement with respect to baseline assessment during chronic treatment but 23 developed dyskinesias, 'off' periods or both, which were difficult to control by changing the levodopa schedule, lisuride dose or both. In only 5 patients was lisuride treatment stopped because of inefficacy. The high incidence of psychiatric side-effects (18/38) was the major limiting factor for continuing lisuride infusion in this population of severe parkinsonian patients. Many complained about the inconvenience of the pump's technical requirements for long-term utilization, but only 4 patients abandoned SLI for this reason. These results indicate that motor function may be substantially improved in severe parkinsonian patients treated with SLI but increased dyskinesia or psychiatric adverse effects limited the long-term usefulness of this approach. It is suggested that continuous dopaminergic stimulation may be a particularly useful therapeutic strategy when applied in earlier stages of the disease.

INTRODUCTION

Daily fluctuations in motor performance and dyskinesias constitute one of the most common and difficult problems to resolve in patients with Parkinson's disease chronically treated with oral levodopa (Marsden and Parkes, 1976). A combination of both pharmacokinetic and pharmacodynamic factors probably are involved in the origin of such complications. Continuous intravenous infusion of levodopa, levodopa methylester or lisuride can reduce or abolish motor oscillations in Parkinson's disease (Cooper *et al.*, 1984; Hardie *et al.*, 1984; Nutt *et al.*, 1984; Quinn *et al.*, 1984; Obeso *et al.*, 1986*a*), suggesting a paramount role of pharmacokinetic mechanisms. However, no difference in the peripheral pharmacokinetics of levodopa or 3-O-methyl-dopa accumulation have been found between patients with and without fluctuation (Fabbrini *et al.*, 1987; Gancher *et al.*, 1987; Luquin *et al.*, 1989). Sustained intravenous levodopa infusion controls the motor state of patients with simple fluctuations ('wearing-off' phenomenon) but only partially stabilizes the motor response in patients with complex oscillations ('on-off' phenomenon) (Marion *et al.*, 1986; Fabbrini *et al.*, 1988). This latter group of patients is also the one showing the highest incidence and severity of

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levodopa-induced dyskinesias (Mouradian *et al.*, 1988). It has been proposed, therefore, that the 'wearing-off' phenomenon essentially arises as a consequence of dopaminergic denervation, while complex 'on-off' fluctuations may indicate additional striatal involvement (Mouradian *et al.*, 1988; Wooten, 1988).

Continuous dopaminergic stimulation has been suggested as a method of stabilizing the motor state in severe parkinsonism complicated by fluctuations (Obeso *et al.*, 1987; Chase *et al.*, 1988). Several drugs and delivery systems are now available for this purpose. Levodopa and levodopa methyl-ester have been successfully used when administered intravenously (Marion *et al.*, 1986; Fabrini *et al.*, 1988; Mouradian and Chase, 1988), by nasogastric route (Kurlan *et al.*, 1986) or after gastrotomy (Sage *et al.*, 1988). Apomorphine and lisuride are very water soluble and therefore easy to dilute in small volumes for subcutaneous administration (Obeso *et al.*, 1986*a*; Stibe *et al.*, 1988). Defining the characteristics of patients most likely to benefit from these new therapeutic approaches is still extremely difficult. In this paper we present the clinical efficacy and factors determining the therapeutic response over a 4 yr period to chronic subcutaneous lisuride infusion (SLI), with oral levodopa, in a large number of patients with severe Parkinson's disease failing to obtain adequate control with standard therapy.

PATIENTS AND METHODS

After informed consent was obtained, 38 patients (26 males, 12 females) with Parkinson's disease (mean age 57.2, range 28–79 yrs) exhibiting fluctuations to L-dopa were admitted to hospital to initiate treatment. All patients had oscillations in motor performance and/or dyskinesias in response to oral levodopa plus dopa-decarboxylase inhibitor after a mean of 9.76 (3–17) yrs of treatment. All had failed to be adequately controlled by adding bromocriptine, selegiline, amantadine and anticholinergic drugs. Fractioning levodopa doses, according to the motor state throughout the day, low protein diet, avoiding ingesting tablets on a full stomach and similar measures had also provided minimal benefit. The King's College Hospital Parkinson's disease disability scale (0–117) was used for clinical evaluation. The motor state situation (including dyskinesias if present), and number of 'on-off' hours were monitored every 30–45 min by 2 of the authors (J.V. and M.R.L.) for 8–10 h daily for 3–5 days before and again after initiation of treatment with subcutaneous lisuride.

Fluctuations were classified as 'simple' (n = 5) when each or most levodopa doses were predictably associated with a short-lasting (1–3 h) 'on' period ('wearing-off'), and 'complex' (n = 33), when the association between levodopa intake and motor function was less predictable (less than 60% of doses induced improvement). Dyskinesias were classified according to their temporal pattern ('on', diphasic and 'off'-period) and type (chorea, mobile dystonia, dystonic postures and repetitive alternating movements). Twenty-six patients had 'on' dyskinesias, 14 showed diphasic dyskinesias and 31 had 'off'-period dystonia (including 8 patients with early morning dystonia). Dyskinesias were scored according to their intensity from 0 (absent) to 5 (severely incapacitating) and duration from 0 (absent) to 5 (continuously present). Night mobility was evaluated by nurses and, during one night, by 1 of the authors, while patients were admitted to hospital, from 0 (normal) to 5 (total immobility).

The values derived from these dyskinetic and nocturnal mobility scoring scales were used for relative comparison with respect to the treatment (infusion) period. After discharge, patients or their relatives filled in 'on-off' charts. Domperidone (10 mg thrice daily, orally) was given to all patients for 3 days before starting the infusion and gradually reduced until discontinuation was tolerated whenever possible. Bromocriptine and anticholinergic drugs were stopped for at least 2 wks before initiating the study. In most cases this did not lead to a significant deterioration of the patient's motor state. Levodopa plus a dopa decarboxylase inhibitor was continued in all patients and adjusted according to clinical response.

Dry lyophilized lisuride (Schering AG, Berlin, Germany) was diluted in 3–3.5 ml of saline for continuous (24 h) subcutaneous administration. Two portable, battery driven, infusion pumps (Tecensa, Madrid, and

Disftronil, Munich) were used for lisuride administration. One of these pumps was able to provide different infusion rates according to a previously established program. In all patients a constant infusion rate was first used, but in a few cases a variable speed was tried later. The initial dose was 0.5 mg/24 h and was raised by 0.5 mg every 3–5 days, as tolerated, until improvement occurred. A 22-gauge needle was placed subcutaneously in the abdomen and initially changed (both needle and site) every 4–5 days, in the first patients, but in most cases replacement was done every 2 days due to the development of nodules at the injection sites. All patients or their spouses were instructed in the use of the pump before discharge. Patients were readmitted for assessment every 3 months during the first year of treatment and subsequently at 6-month intervals, or any time that side-effects occurred.

In 8 subjects, hourly lisuride concentrations were measured for 24 h before discharge and 12 months later. Blood was collected into heparinized tubes, plasma separated and stored at −70° C until assayed by radioimmunoassay as previously described (Hümpel *et al.*, 1981). Routine biochemical and haematological tests were performed before starting lisuride, at the time of discharge and every 3 months during the first year. An ECG was taken at similar intervals.

Paired t tests and Wilcoxon tests for nonparametric data were used for statistical analysis. Results are expressed as the mean ±1 SD and range when appropriate.

RESULTS

Initial results

Fluctuations. Thirty-eight patients were treated with continuous SLI, mean dose 111.3 ± 29.55 μg/h, added to oral levodopa-DDI (729.6 ± 452.07 mg/day) during a mean hospitalization period of 5 wks (range 4–8). Two patients had treatment withdrawn during their initial admission to hospital because of psychiatric adverse effects. These 2 cases are not included in the evaluation of the effect of lisuride infusion on motor function. In the remaining patients, motor function at the time of initial discharge showed a significant improvement ($P < 0.001$) compared with preinfusion assessments (fig. 1). In 14 patients (5 with simple and 9 with complex fluctuations), 'off' periods were completely abolished with no increment in dyskinesias. In the remaining 22 patients with complex fluctuations, the periods of reduced mobility persisted but the severity of such 'off' states had lessened (fig. 1; $P < 0.001$), allowing a wider time interval between doses (Table 1).

The main daily dose of levodopa was decreased during initial hospitalization (Table 1), but levodopa could not be eliminated in anyone. The number of daily doses of levodopa was also reduced from a mean 6.2 ± 2.1 to 4.5 ± 0.61 ($P < 0.05$). In this situation every levodopa dose was reliably accompanied by a positive effect on motor performance. The feeling of independence and confidence at that time was perhaps the most striking, albeit nonquantitative, effect of the infusion in most patients. Unexpected 'off' periods were recorded in 18 patients. In every instance this was due to technical failure of the infusion pump mechanism. All patients returned to their normal therapeutic response within a few hours of reestablishing an adequate infusion rate. Movement capacity during the night also improved substantially (Table 1). In general, clinical improvement started to occur some 7–10 days after starting SLI, but maximal benefit was seen only several weeks after treatment initiation (mean 7.5 ± 1.2 wks).

Dyskinesias. Dyskinesias during 'on' periods (mainly chorea) were reduced in 11 patients, unchanged in 9, increased in 5 and appeared for the first time in 4. Diphasic dyskinesia were reduced in 9, unchanged in 2, increased in 2 and appeared in one; 'off' period dystonia was abolished in all 30 patients.

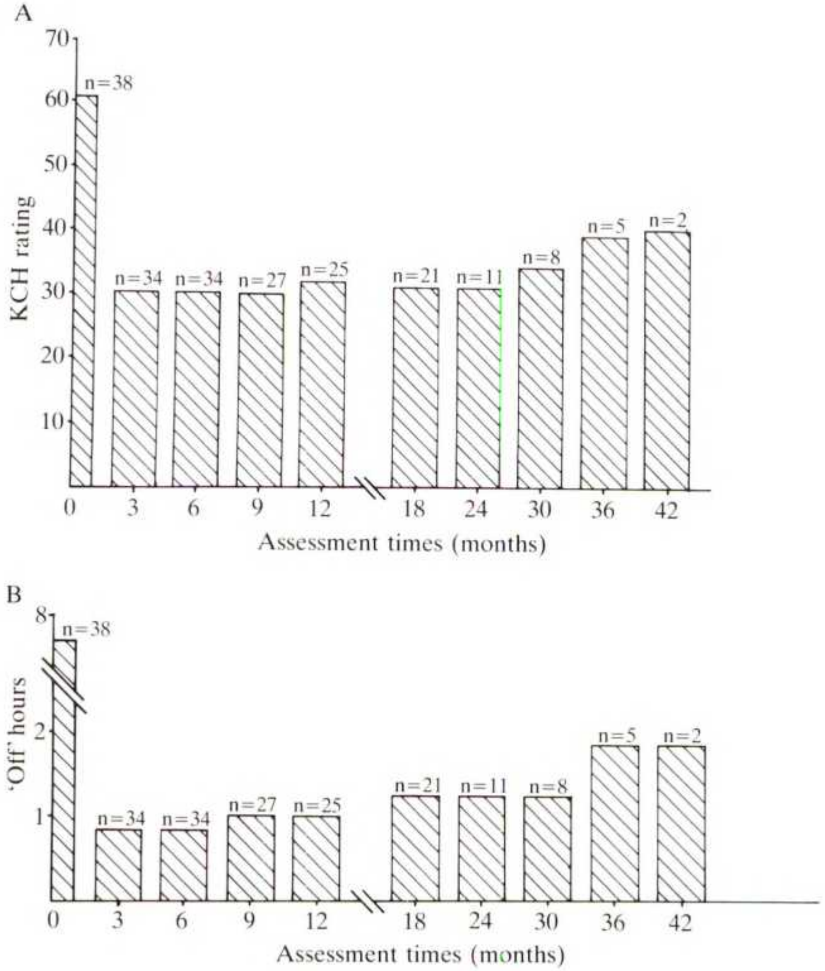

FIG. 1. Temporal evolution of motor disability (A) as evaluated by King's College Hospital (KCH) Parkinson's disease rating scale and number of daily 'off' hours (B) from 8 a.m. to 8 p.m. The numbers of patients receiving SLI and levodopa plus a decarboxylase inhibitor are given above the columns. Times of assessment during treatment are given in months.

Follow-up

Thirty-four patients continued treatment with SLI (mean 109 ± 26.5 μg/h) added to oral levodopa-DDI (719 ± 408.8 mg daily) during a mean period of 20.85 months (range 6–45). One patient discontinued SLI within the first 3 months of treatment due to psychiatric side-effects and another could not adequately control the pump-mechanism. Fig. 1 summarizes the evolution of 'on-off' and disability when 'off' throughout a mean treatment period of 20.85 months. The initial improvement was maintained in most cases (fig. 1A, B), but the number of patients under SLI treatment decreased markedly (26 patients dropped out during the first 2 yrs; 13 each yr) due to several reasons (Table 2). Motor fluctuations and severity of 'off' periods was significantly improved with respect

TABLE 1 EFFECT OF SUBCUTANEOUS LISURIDE INFUSION IN PATIENTS WITH MOTOR FLUCTUATIONS

	Preinfusion (n = 36)	*Initial response*[3] *(n = 36)*	*Last assessment*[4] *(n = 27)*	*Patients who currently continue treatment (n = 7)*
Treatment period	–	5 wks	20.11 mos (6–45)	27.15 mos (20–38)
Levodopa+DDI[1] (mg/day)	1038.67±382.5	729.6±452.7	784.7±306.8	401±323.2*
Lisuride (μg/h)		111.3±29.55	109.9±26.5	100.8±25.2
'Off' h/day	7.3±1.5	0.86±0.9*	1.5±1.3*	2±1.5*
Motor scale[2]				
'On'	19.1 ±7.8	16.6±6.9	16.4±7.4	27.4±12.4
'Off'	61.1±16	30.7±14.6*	34.6±14*	45.5±8.3*
Night mobility	3.7 ±0.7	1.5±0.8*	2±0.4**	2.7±1.2
No. of daily doses	6.2 ±2.1	4.5±0.6*	4.6±1.2*	4±1.6*

[1] DDI = dopa decarboxylase inhibitor. [2] King's College Hospital Disability Scale (range 0–117). [3] At the time of first discharge [4] Data taken at the time of discontinuing LSI for reasons summarized in Table 2, or during follow-up after a minimum treatment period of 6 months (mean 20.85, range 6–45). The asterisks indicate statistically significant differences with respect to preinfusion values (*$P < 0.001$, **$P < 0.05$).

TABLE 2 REASONS TO STOP SUBCUTANEOUS INFUSION OF LISURIDE (SLI) (n = 29) DURING CHRONIC TREATMENT IN 36 PATIENTS

	Duration of treatment (mos)						
	3	*9*	*12*	*18*	*24*	*36*	*48*
Reasons for stopping SLI							
Psychiatric reaction (n = 16)	1	4	1	1	6	3	–
Dissatisfaction, desired different treatment (n = 5)	–	1	–	–	2	1	1
Therapeutic inefficacy (n = 5)	–	1	4	–	–	–	–
Unable to handle the technology of the pump (n = 1)	1	–	–	–	–	–	–
Infected nodules (n = 1)	–	–	–	–	–	–	1
Death* (n = 1)	–	–	–	–	–	–	1
Currently continuing SLI (n = 7)	–	–	–	3	2	2	–

* Unrelated to drug treatment.

to baseline assessment in the great majority of patients up to the time of discontinuation (Table 1). Choreic dyskinesias increased over the months of treatment (fig. 2). At the time of last assessment chorea had been increased in 23 patients, it was unchanged in

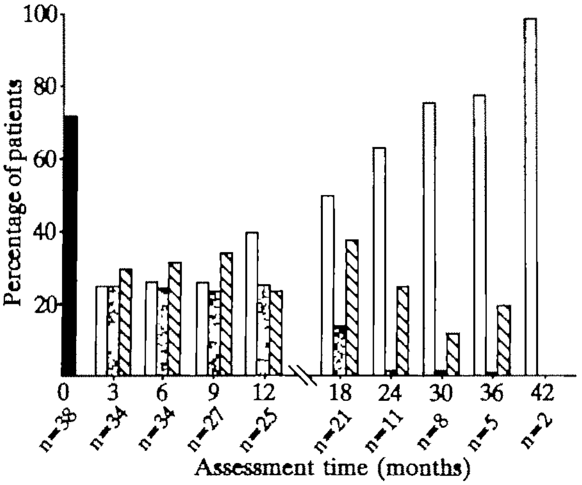

FIG. 2. Temporal evolution of chorea after initiating treatment with SLI and levodopa plus a decarboxylase inhibitor. The proportion of patients showing clinically relevant chorea (score 2 or above in the dyskinesia scale) before starting SLI is shown by the black bar to the left. The proportion of patients in whom chorea increased or it was reduced by at least 25% with respect to baseline assessment (black bar) is shown in white and dotted bars, respectively. Patients with chorea of new onset are shown by hatched bars.

6 and reduced in 5. One patient stopped SLI because of unbearable chorea (score 5). Diphasic dyskinesia increased in 8 patients leading to SLI discontinuation in 2 patients. An adequate control of 'off' period dystonia was maintained in most patients throughout treatment.

Therapeutic profile of SLI

The above data indicate that the motor response was maintained, but dyskinesias were enhanced in most patients during treatment with SLI and levodopa-carbidopa (orally). However, the actual therapeutic capability of this strategy is not fully represented in that analysis. Accordingly, the degree of benefit compared with complications over long-term treatment can be summarized in 3 groups of patients:

Group A. Excellent response. Patients (n = 11) who maintained the initial good response with no 'off' periods throughout the day or only mild and transient deteriorations in motor capability. Dyskinesias were not enhanced in this group. Motor disability was dramatically improved, so that they returned to their normal activities. Three patients of this group had 'simple fluctuations' and 8 'complex fluctuations' before lisuride treatment. These 11 patients were treated for a mean period of 20.9 ± 11.1 months (range 6–45). The daily dose of levodopa (plus a dopa decarboxylase inhibitor) was reduced by 47% in this group. In 6 patients SLI was stopped because of psychiatric side-effects after 17.16 (range 6–33) months. Another patient with chronic bronchitis died from pneumonia unrelated to Parkinson's disease after 45 months of SLI. One patient decided to look for a different therapeutic approach. Three patients continue at present on treatment with SLI after a mean period of 25 (range 21–28) months.

Group B. Increased management difficulty. Eighteen patients (2 with 'simple' and 16 with 'complex' fluctuations) in whom 'off' periods were reduced (totally abolished in 2 patients) in severity and duration during the 25.36 ± 7.3 (range 10–43) months of treatment, but choreic 'on' dyskinesias increased by more than 50% with respect to the initial evaluation. The daily requirement for levodopa (with a dopa decarboxylase inhibitor) was reduced by a mean of 32%. Every attempt to modify the daily levodopa dose and schedule or changes in lisuride infusion regimen invariably led to an intolerable reappearance or worsening of 'off' periods.

Despite these therapeutic problems the 18 patients of this subgroup remained subjectively and objectively improved as compared with the control period up to the end of treatment, because motor fluctuations were predictable and disability during 'off' periods was milder. Nine patients discontinued lisuride infusion after 20.5 (range 7–32) months because of psychiatric side-effects. Four patients decided to try other treatments. One patient developed very severe nodules at the injection site after 43 months of treatment. The remaining 4 patients continued treatment at present after 28.5 ± 6.5 (range 20–38) months.

Group C. Therapeutic failures (n = 5). These patients with 'complex' fluctuations improved initially, maintaining the same daily dose of levodopa, but over the next 10.6 months of SLI therapy (range 6–15) severe 'off' periods returned. The daily dose of lisuride was subsequently increased and the rhythm modified with the intention of providing a higher dopaminergic stimulus during the most conflictive hours of the day (fig. 3). Two patients developed psychotic episodes while the other 2 experienced prolonged and violent dyskinesias in the lower limbs for many hours, causing physical exhaustion and intense emotional distress. The other patient had severe 'off' periods which did not respond to SLI plus levodopa. The infusions were discontinued in all 5 patients.

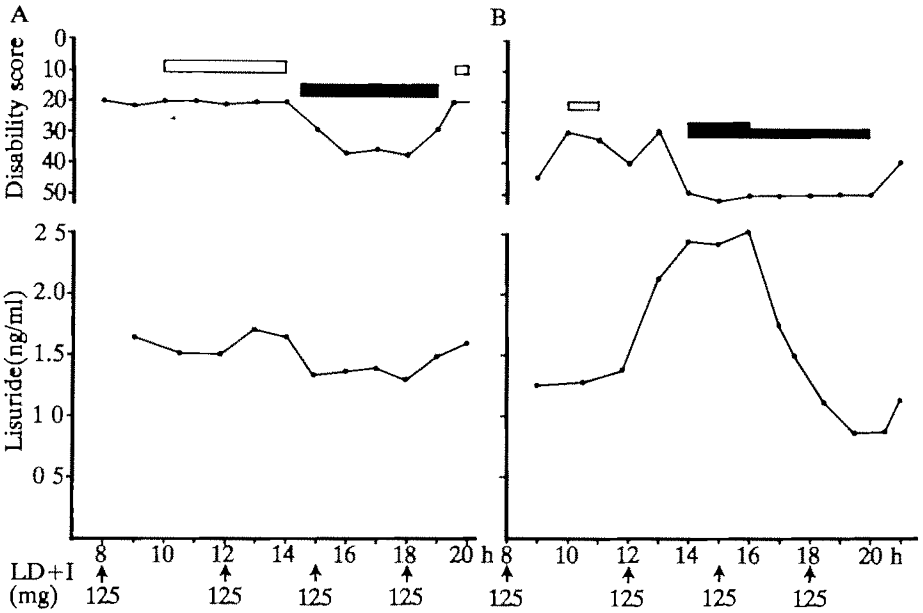

FIG 3 Patient S C. showed mild episodes of intermediate mobility (A, *top*), accompanied by repetitive alternating movements in the lower limbs (RAMs) in the afternoon while receiving a constant lisuride infusion (A, *bottom*). The motor situation deteriorated markedly (B, *top*) when lisuride infusion rate was changed to provide higher but more variable plasma levels (B, *bottom*) LD+I = levodopa plus dopa decarboxylase inhibitor Open bars = Chorea; filled bar = dystonia; hatched bar = RAMs

In summary, 7 patients, 3 from group A and 4 in group B, continue with SLI at present after a mean treatment period of 27.15 ± 8.2 (range 20–38) months. Psychiatric side-effects were the main reason for discontinuing SLI (Table 2). Most patients who continued for over 2 yrs of treatment complained about the demanding nature of the method, but only 4 (from group B) actually became disaffected and decided to seek alternative treatment.

Plasma levels

Lisuride levels were constant during the 24 h cycle (fig. 4). Plasma lisuride concentrations were measured in 8 patients (3 from group A, 4 from group B, 1 from group C) at initial discharge and after a mean of 12 months of follow-up. No difference in plasma steady-state levels of lisuride or area under the curve were observed among patients belonging to different groups over time ($P > 0.05$), which could explain the differences in motor response.

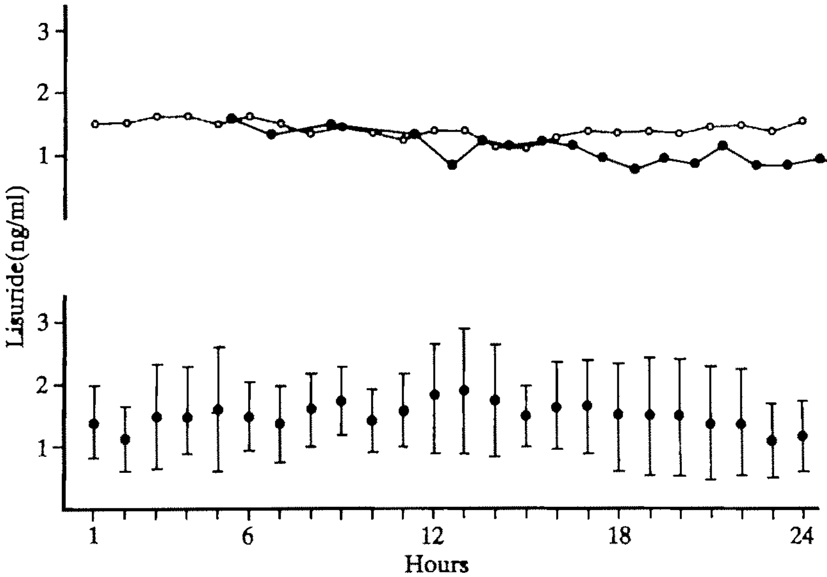

FIG. 4 *Upper half*. Lisuride plasma levels in a patient treated with subcutaneous lisuride infusion (2.5 mg/24 h). Open circles indicate plasma values at the beginning of treatment and closed circles 1 yr later. *Lower half*. Mean lisuride plasma levels in 8 patients treated with continuous subcutaneous lisuride infusion (mean dose 2.35 mg/24 h). Bars represent 1 SD.

Side-effects

On beginning the infusion, 9 patients developed nausea and vomiting which gradually improved over the next few days. Fifteen patients complained of an ill-defined sense of discomfort or malaise; 5 patients developed pronounced sweating diaphoresis. Five subjects noticed temporary frontal nonpulsatile headache requiring occasional analgesic treatment. One patient tolerated the infusion well but could not control the pump-mechanism adequately and was dropped from the study during the first month.

Fourteen patients developed nodules at the injection sites of sufficient size to cause attention or give rise to discomfort, but smaller nodules were present in practically every patient. Three patients developed on one occasion each a local abscess which required antibiotic therapy and surgical incision in one. In this latter case a second subcutaneous infection led to discontinuation of SLI. Biopsy of the nodules revealed local nonspecific inflammatory changes. Nodule formation was accompanied by a transient deterioration in motor performance due to reduced lisuride absorption (unpublished data). This local complication led us to instruct the patients to change the needle and insertion site every 2 days, a manoeuvre which dramatically reduced the presence of nodules. Nine male patients experienced weight gain (10 ± 1.7 kg over the first 6 months of treatment). Most male patients admitted to mildly increased libido. Four patients complained daily of nonmigrainous headache over several months of treatment. Two patients became stuporose when a large amount of lisuride (2.5 mg in 1 h) was accidentally delivered due to a breakdown in the pump's infusion mechanism. Both recovered spontaneously within 24 h without any specific treatment.

The major complication limiting SLI consisted of adverse psychiatric reactions. Two patients developed visual hallucinations evolving into frank pyschosis during the initial admission and were dropped from the study. Sixteen patients had psychiatric symptoms over a mean treatment period of 13 (range 2–45) months. Nightmares and vivid dreams were present at some stage of treatment in all 17 patients. In 12, a severe psychosis developed. This was preceded in every patient by irritability, increased perception of external stimuli, delusions, visual and auditory hallucinations and finally paranoia. This sequence of events had a variable time evolution, ranging from hours to 1–2 wks. In these 12 patients, lisuride infusion was discontinued for this reason. The other 4 patients developed isolated visual hallucinations which disappeared when lisuride infusion was interrupted for a few hours. However, SLI had finally to be abandoned in these 4 cases due to recurrence of visual hallucinations after 21.25 months of treatment (range 12–34). In 3 of these patients hallucinations seemed to be triggered by an oral dose of a benzodiazepine. In 1 patient the lisuride daily dose had to be reduced four times, over a total period of 30 months, from 2.5 mg/24 h initially to 1 mg/24 h on the last admission, due to recurrent episodes of visual hallucinations. Following each reduction in lisuride dosage, plasma levels decreased accordingly, and motor function slowly deteriorated.

Analysis of clinical factors (i.e., disease duration, years and dose of levodopa, disease severity, etc.), possibly conditioning the appearance of pyschiatric side-effects, did not reveal any difference between patients developing such complications and those who continued to tolerate lisuride infusion.

Routine biochemical tests in plasma and urine, including liver and kidney function, were repeated every 3 months, but failed to show any abnormality. Platelet aggregation was first studied in a patient who developed generalized spontaneous haematomas of small size. Subsequently platelet aggregation was found to be altered in 90% of patients, consisting of a desegregation-hypoaggregation with ADP, though in some patients (n = 18) there was a global hypoaggregation (ADP, adrenaline and collagen). This had no clinical significance in any of the patients, except for 3 who had cutaneous haematomas after small injuries at the site of the insertion of the needle in the abdominal wall.

DISCUSSION

In 34 parkinsonian patients with levodopa-induced motor fluctuations and dyskinesias, SLI produced a considerable improvement of disability which was sustained over many months of treatment. The characteristics of this form of treatment and the severity of the patients made the design of a double-blind controlled study very difficult. The possibility of a placebo effect contributing to the initial striking improvement cannot be excluded entirely. Nevertheless, occasional interruptions of the infusion due to inadequate pump function provided unplanned opportunities to check the true therapeutic effect of this treatment. More importantly, there was no clear clinical (Table 1, fig. 1) or pharmacological (Vaamonde *et al.*, 1990) evidence that a phenomenon of tolerance developed during chronic lisuride infusion.

Probably the most striking clinical effect seen in the patients treated with SLI was the modification of motor fluctuations from a complex to a simple pattern, where the effect of each levodopa dose became predictable. Many different dose schedules and measures to improve levodopa absorption have been tried before in these patients without marked benefit. The possibility that changes in levodopa pharmacokinetics would explain this therapeutic response seems therefore very unlikely. Lisuride infusion appears to have provided sufficient dopaminergic stimulation to minimize motor incapacity during levodopa interdose periods; this in turn allowed an increase in the time intervals between doses which probably also plays a role in assuring the efficacy of each levodopa dose (Nutt, 1988; Grandas and Obeso, 1989). The need for concomitant use of levodopa in all our patients was unexpected since lisuride shows a very high affinity for dopamine receptors in vitro (Fujita *et al.*, 1979), and lisuride plasma levels were maintained constant. In the past (Obeso *et al.*, 1986*b*), these findings were tentatively explained on the basis of the selective action of lisuride on D2 receptors. However, fluctuating parkinsonian patients treated with subcutaneous infusion of apomorphine, a mixed D1-D2 agonist, also required supplementary oral levodopa (Obeso *et al.*, 1987; Stibe *et al.*, 1988). In contrast, Stocchi *et al.* (1988) observed very good control of motor fluctuations with subcutaneous lisuride alone in 6 patients. These patients had less severe disease than those included in our study. One explanation, therefore, is that the therapeutic action of dopamine agonists delivered continuously may require the regulatory influence of presynaptically released dopamine; this could either be provided endogenously from surviving nigrostriatal terminals in less severe patients or from exogenously administered levodopa in more advanced cases. In agreement with earlier studies using intravenous or duodenal levodopa infusion (Hardie *et al.*, 1984; Nutt *et al.*, 1984; Quinn *et al.*, 1984; Kurlan *et al.*, 1986, 1988) the initial marked benefit obtained in our patients could be taken as evidence of relatively preserved striatal dopaminergic responsiveness in Parkinson's disease, hence suggesting a paramount role of pharmacokinetic factors in the origin of levodopa-related motor oscillations (Kurlan *et al.*, 1988; Riley and Lang, 1988). However, several observations in our large group of patients chronically treated with SLI also indicate a relevant participation of pharmacodynamic mechanisms. Lisuride plasma levels remained constant throughout many months of treatment, but the motor response changed with time. Dyskinesias became a major troublesome complication in an important proportion of cases. 'Peak of dose' chorea increased in about 50% of patients, suggesting the emergence of dopaminergic hypersensitivity (Klawans *et al.*, 1979). On the other hand, a few patients developed a complex dyskinetic pattern characterized by generalized chorea and full mobility in the morning, and repetitive movements of the lower limbs, associated with an intermediate motor state, during many hours in the afternoon. The latter movements are characteristic of those seen in 'diphasic dyskinesias', but in these cases they lasted for many hours. It seems as if, in such instances, lisuride infusion provided a sufficient dopaminergic (DA) stimulus to trigger such movements, but was incapable of achieving a complete 'on' state, thus perpetuating the dyskinetic situation. Whether the appearance of such dyskinesias when a given threshold of DA stimulation is reached, as suggested by our findings, is due to selective activation of a subgroup of DA receptors or some other putative mechanism is not known. Patients suffering this complication were those requiring the highest daily levodopa and lisuride dose and, in acute tests, they failed to improve after subcutaneous apomorphine administration during the afternoon episodes (Vaamonde *et al.*, 1989) suggesting the existence of decreased striatal dopamine receptor sensitivity. Regarding the motor response to SLI the larger experience presented in this paper confirms our preliminary observations (Obeso *et al.*, 1986*b*, 1988). However, the therapeutic advantages after long-term treatment are not strikingly convincing in this population of patients. We believe, nevertheless, that different patterns of response may be obtained in patients treated with SLI and oral levodopa. The most stable and adequate control was achieved in patients with simple oscillations who had milder 'off' episodes and in whom levodopa dose could be substantially reduced after starting lisuride infusion. In contrast, therapeutic failures or unstable responses were observed in patients with complex oscillations, severe 'off' episodes and those who required high levodopa dosages despite lisuride infusion. This is in keeping with the findings of Fabbrini *et al.* (1987) in patients receiving intravenous levodopa infusion for several consecutive days, and supports their arguments for pharmacodynamic differences in the pathophysiology of simple ('wearing-off') and complex ('on-off') fluctuations. However, the difficulty in maintaining a stable motor response over time with SLI treatment argues against the possibility of reversing the presumed postsynaptic abormalities by means of continuous dopaminergic stimulation (Fabbrini *et al.*, 1988). Admittedly, lisuride may not provide optimum dopaminergic stimulation and perhaps better long-term results could be achieved with other dopamine agonists, such as apomorphine (Stibe *et al.*, 1988), +PHNO (+4-propyl-9-hydroxinapthoxazine) or levodopa itself (Kurlan *et al.*, 1986; Sage *et al.*, 1988). Differences in the delivery regimen and time under treatment of patients treated with these drugs at present preclude accurate comparison with our data.

In addition to the changes in motor response, adverse psychiatric reactions constituted another major limiting factor to lisuride infusion. Such psychiatric manifestations had been encountered previously in parkinsonian patients treated with oral lisuride and with other dopaminergic drugs, including some without central serotoninergic activity (Horowski, 1986). We believe that the constancy of the stimulus (24 h) and the high lisuride plasma levels achieved by subcutaneous administration may explain the high incidence and striking intensity of such adverse effects. Indeed, none of the 12 patients chronically treated by Stocchi *et al.* (1988) with 12 h subcutaneous infusions developed significant psychiatric abnormalities, while the opposite was the case for the more severe parkinsonian patients, many of whom had a previous history of drug-induced hallucinations, studied by Critchley *et al.* (1988). It may therefore be that the mode of delivering lisuride (and other agonists) and the functional state of the dopaminergic systems could have an important influence on its pharmacological effect. In this regard, it is interesting that evaluation of motor function and dopaminergic response in the patients who developed psychiatric symptoms did not reveal any difference, either before or after lisuride treatment. Such findings would suggest a dissociation between the biochemical basis of motor behaviour and the pyschiatric reactions.

Traditionally levodopa has been given discontinuously, in pulses, throughout the day. This produces wide fluctuations in synaptic dopamine disposition and possible unphysiologically high synaptic levels of the neurotransmitter (Melamed, 1988). These factors could be partially responsible for the motor complications associated with chronic levodopa use since neurons of the sustantia nigra pars compacta are normally characterized by low-rate tonic discharges, with minimal variations during movement, resting or even sleeping conditions (DeLong *et al.*, 1983; Schultz *et al.*, 1983). Whether or not continuous dopaminergic stimulation is a more appropriate approach to treating Parkinson's disease is difficult to ascertain at present. In the few patients where changes in the infusion rhythm throughout the day were attempted, motor fluctuations and dyskinesias became worse, suggesting that continuous lisuride delivery provides a more physiological form of striatal stimulation. On the other hand, the high incidence of psychiatric reactions could be directly related to the 24 h infusion regimen we have used. The therapeutic possibilities of lisuride infusion and perhaps also of other water soluble DA agonists will depend mainly upon this problem. Most of our patients had long-standing and severe Parkinson's disease. Tolosa *et al.* (1989) recently found a low incidence (12%) of psychiatric complications in 18 patients with mild to moderate Parkinson's disease who, unlike our patients, required small subcutaneous lisuride doses in 24 h. We therefore suggest that continuous dopaminergic stimulation could provide optimal therapeutic results when applied to relatively mild patients. This requires appropriate investigation to be validated or refuted.

ACKNOWLEDGEMENTS

This work was partially supported by a grant from the Spanish Government (CAYCIT), Dr Luquin and Dr Vaamonde were supported by a research grant from Schering AG, Lisuride plasma levels were kindly assayed by Dr B. Nieuweboer and W. Krause (Schering, Berlin). The authors are grateful to Drs R. Horowski, A. Lang and S. Gancher for helpful suggestions on this paper. Mrs M. M. López and Ms C. Elsden edited the manuscript.

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(Received October 18, 1989. Revised February 16, 1990. Accepted April 15, 1990)

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MOTOR REORGANIZATION AFTER UPPER LIMB AMPUTATION IN MAN

A STUDY WITH FOCAL MAGNETIC STIMULATION

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SUMMARY

To evaluate reorganization in motor pathways following amputation, we studied motor evoked potentials (MEPs) to transcranial magnetic stimulation in 7 patients with unilateral upper limb amputations, a patient with congenital absence of a hand, and 10 normal subjects. Electromyographic recordings were made from biceps and deltoid muscles immediately proximal to the stump and the same contralateral muscles. Magnetic stimulation was delivered by a Cadwell MES-10 magnetic stimulator through a 'figure eight' magnetic coil over scalp positions separated by 1–2.5 cm. Maximal M responses were elicited by peripheral nerve stimulation at Erb's point. The amplitude of MEPs was expressed both as absolute values and as a percentage of maximal responses to peripheral nerve stimulation. Threshold for activation of muscles ipsilateral and contralateral to the stump and the region of excitable scalp positions were also determined in 7 patients.

Magnetic scalp stimulation induced a sensation of movement in the missing hand or fingers in the patients with acquired amputation, but failed to do so in the patient with congenital absence of a limb. It evoked larger MEPs, recruited a larger percentage of the motoneuron pool, and elicited MEPs at lower intensities of stimulation in muscles ipsilateral to the stump than in contralateral muscles. Muscles ipsilateral to the stump could be activated from a larger area than those contralateral to the stump. These results are compatible with cortical or spinal reorganization in adult human motor pathways targeting muscles proximal to the stump after amputations.

INTRODUCTION

Amputation of a limb involves damage of all nerves supplying the lost segment. Anatomically, it is associated with retrograde changes above the amputation site at different levels. The stump contains multiple neuromas and regenerating mature and immature axons growing irregularly into the surrounding connective tissue (Sunderland, 1978). The nerve trunks and roots serving the stump, the anterior and posterior horns and dorsal column ipsilateral to the stump atrophy (Bérard, 1829; Ranson, 1906). The cerebral cortex neighbouring the contralateral central sulcus has been reported as atrophied (Raymond, 1882; Campbell, 1905), although the causal relationships of the amputation with the atrophy have not been established. This macroscopic observation has been well documented (*see* Sutherland, 1978, for review).

From a functional point of view, it was suggested as early as 1923 that certain patterns

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in the organization of the human cerebral cortex (i.e., motor cortex) could be 'temporary' (Lashley, 1923). Many years later, reorganization in animals was demonstrated in visual (Hubel and Wiesel, 1970), somatosensory (Kaas *et al.*, 1983), olfactory (Wilson *et al.*, 1987), and motor (Donoghue and Sanes, 1987) areas after peripheral manipulations. These changes were particularly evident in early stages of development, but also were demonstrated in adulthood (Wall and Cusick, 1986). An exciting conclusion of these experiments is that the organizational patterns observed in the cerebral cortex are not static, but can change under certain conditions.

Human studies addressing these issues have presented obvious difficulties because of the invasive nature inherent to the techniques used. In the last few years, however, there have been attempts to evaluate changes in the human somatosensory system after amputation by using somatosensory evoked potentials (SEPs) (McComas *et al.*, 1978*a*, *b*; Sica *et al.*, 1984, 1988). It has been suggested that 'SEP generators previously devoted to the limb segment which was amputated, may be invaded by inputs coming from intact areas located at the same side of the amputation' (Sica *et al.*, 1988).

The question we address here is whether there are any changes in the map of outputs of the adult human motor cortex after amputation. The technique of noninvasive, painless magnetic stimulation of the brain (Barker *et al.*, 1985) provides a useful tool for exploring this possibility. The use of magnetic coils that optimize focality has improved the spatial resolution of the technique (Cohen *et al.*, 1989, 1990*a*). A preliminary account of these observations has been published previously (Cohen and Hallett, 1989).

METHODS

We studied 7 patients (4 women and 3 men), aged 24–78 (mean 47) yrs, with unilateral upper limb amputations of 1–12 yrs' duration; 1 woman with congenital absence of a hand, aged 57 yrs; and 10 normal volunteers (3 women and 7 men), aged 25–70 (mean 37) yrs (Table 1). Neurological examination was normal All subjects gave written informed consent for participation in the study, and the protocol was approved by the Institute's clinical research subpanel.

TABLE 1 CLINICAL CHARACTERISTICS OF THE PATIENTS

Case	*Age (yrs)*	*Sex*	*Amputation*		*Muscles studied*
			Level	*Date*	
1	57	F	L below elbow	Congenital	Biceps
2	52	M	R elbow	1977	Biceps
3	65	F	L above elbow	1988	Deltoid
4	30	M	R above elbow	1985	Deltoid
5	78	F	L elbow	1988	Biceps
6	24	F	L above elbow	1985	Deltoid
7	25	M	L above elbow	1985	Deltoid
8	45	F	R below elbow	1987	Biceps

Stimulating techniques

Transcranial magnetic stimulation was delivered by a Cadwell MES-10 magnetic stimulator through a 'figure eight' magnetic coil while subjects were at rest. The magnetic coil has two circular sides, each 4.5 cm in diameter. The intersection of the sides (centre) is approximately 3 cm long × 1 cm wide, and the current in the centre initially flows towards the handle. The magnetic coil was positioned on the scalp with the handle along the sagittal axis and its centre flat on the target scalp position. Thus the coil centre was in contact with the scalp and closer to the target neural tissues than the sides. Theoretical calculations and physiological studies show that the electrical field induced by this coil is maximal beneath the centre (Cohen *et al.*, 1989, 1990*b*; Bandinelli *et al.*, 1989). For mapping of motor evoked potentials (MEPs), stimuli were delivered randomly to different scalp positions located 1 or 2.5 cm apart.

Electrical and magnetic stimuli were delivered to the brachial plexus at Erb's point for biceps brachii and deltoid muscles to determine the maximal M responses, the largest responses evoked by either technique to peripheral nerve stimulation. Both techniques were used in every normal subject. Since electrical stimulation was painful for 3 patients, only magnetic stimulation was used for them.

Recording techniques

Electromyographic (EMG) responses were recorded by tinplate surface electrodes (DISA 13K60) overlying the biceps or deltoid muscles immediately proximal to the stump and from the same muscles contralaterally. Recordings were made from the biceps in 4 patients and from the deltoid in 4 others (*see* Table 1). Signals were amplified with a DISA 1500, using a bandpass of 100–2000 Hz, and recorded over 50 ms. The amplitude of peripheral M responses was measured peak-to-peak. The amplitude of MEP to transcranial stimulation was measured peak-to-peak and expressed both as absolute values (μV) and as a percentage of maximal M responses to peripheral nerve stimulation. In this manner, we obtained an approximation of the percentage of the motoneuron pool activated by scalp stimulation. Three muscle responses to stimulation of each scalp position were recorded and the average of the three responses was assigned to represent the scalp position stimulated.

Mapping of MEPs

Different scalp positions overlying sensorimotor areas, separated by 1 cm along the coronal axis (7 patients) and 2.5 cm along the coronal and sagittal axes (6 patients) were stimulated (Fig. 1). Stimuli of equal intensities, 100% of the output of the magnetic stimulator, were delivered randomly to each of these positions on both sides of the scalp until each position received at least 3 stimuli. A side-to-side ratio was calculated for the number of excitable scalp positions (positions from which muscle responses of at least 100 μV were elicited from the muscle proximal to the stump). In the amputees (14 muscles in 7 patients), the ratio was between the size of the motor representation area corresponding to the muscle ipsilateral to the

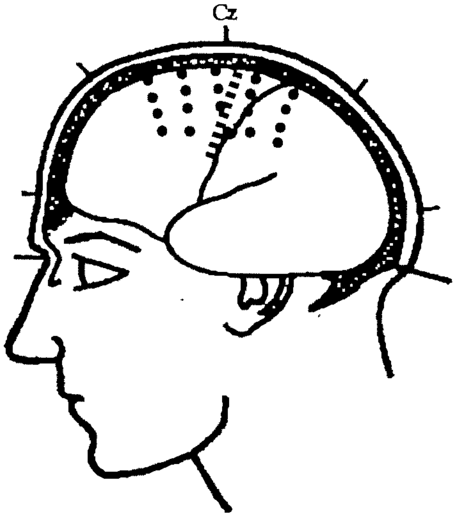

FIG 1. Scalp positions stimulated Positions along the coronal and sagittal axes were separated by 2.5 cm (circles) and positions along the coronal axis, across Cz, were separated by 1 cm (squares) The 1 cm distance between stimulating positions allowed better spatial resolution in the critical coronal axis

stump and the size of the area corresponding to the muscle contralateral to the stump. In the controls (20 muscles in 10 subjects), the ratio was between the largest of the two motor representation areas and the smallest. The latencies of the MEPs were measured, and latency-position curves were constructed to compare the latencies of MEPs as a function of the scalp position stimulated in each patient on both sides.

Thresholds for muscle activation

The centre of the magnetic coil was positioned over the optimal scalp locations for stimulation of each muscle as determined in the previous experiment. The lowest intensity of stimulation capable of inducing a 100 μV muscle response from the muscle immediately proximal to the stump ipsilaterally and contralaterally in at least 5 out of 10 trials in 7 patients was determined.

Statistical analysis

Side-to-side comparisons in the amputees were performed with paired t tests. Comparisons of amputees and normal subjects were performed with unpaired t tests. Variance is expressed as the SEM. Results are adjusted for determination of experimentwise error (Cohen and Cohen, 1975). The significance level is $P \leq 0.01$ to adjust for multiple t tests.

RESULTS

Magnetic scalp stimulation induced a sensation of movement in the missing hand in the 7 patients with acquired amputations, but failed to do so in the patient with congenital absence of a hand. In all patients, stimuli activated muscles in the stump.

Muscles ipsilateral to the stump were activated from more numerous scalp positions (3.00 ± 0.45 and 3.71 ± 0.42) than those contralateral to the stump (1.33 ± 0.21 and 1.85 ± 0.45) for distances 2.5 and 1 cm apart, respectively ($P \leq 0.01$ and ≤ 0.006) (figs 2, 3). The side-to-side ratio for the number of excitable scalp positions at 1 cm apart along the coronal axis was larger in the amputees than in the controls (2.58 ± 0.55 and 1.29 ± 0.06, respectively, $P \leq 0.01$) (fig. 4).

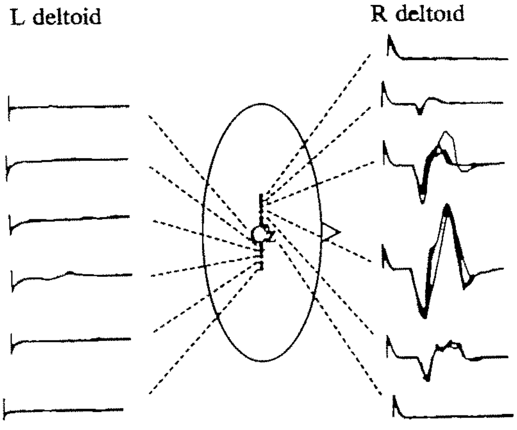

FIG. 2. Schematic diagram of the top of the head of *Case 4* who had amputation of the right arm above the elbow 4 yrs before. EMG responses were evoked by stimulation of different scalp positions 1 cm apart along the coronal axis with equal stimulus intensities. MEPs on the left side of the scalp were recorded from the right deltoid and those on the right side of the scalp were recorded from the left deltoid. Note that stimulation of 4 positions evoked responses from the deltoid ipsilateral to the stump (*right*), while stimulation of only one position evoked responses from the deltoid on the intact side (*left*). Calibration bar = 5 ms and 100 μV.

Latencies of MEPs ipsilateral to the stump were generally shorter than latencies of MEPs contralateral to the stump (Table 2), although in Case 6, stimulation at different scalp positions elicited both shorter and longer latencies of ipsilateral MEPs relative

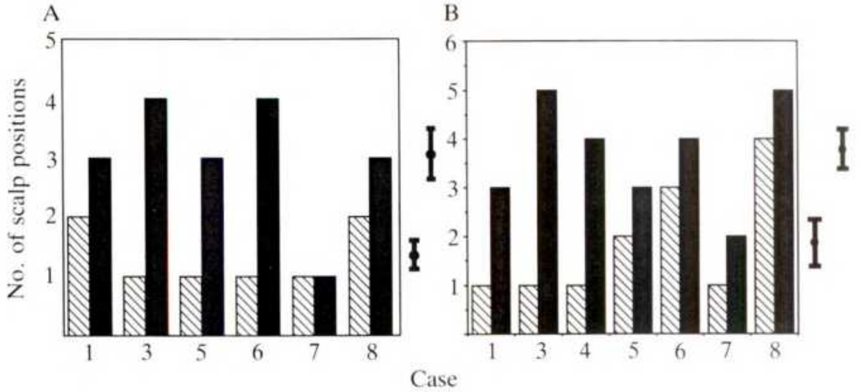

FIG. 3. Number of excitable scalp positions for muscles ipsilateral (solid bars) and contralateral (hatched bars) to the stump when stimuli of equal intensities were delivered to positions separated by 2.5 cm (A) and 1 cm (B). Means ± SEM are shown on the right of each graph.

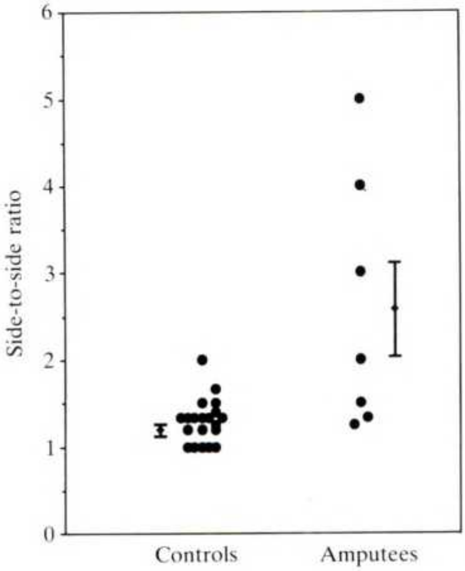

FIG. 4. Side-to-side ratio for the number of excitable scalp positions in 20 controls (10 biceps and 10 deltoids) and 7 amputees.

to MEPs contralateral to the stump. Stimulation of optimal scalp positions activated muscles ipsilateral to the stump at lower thresholds than muscles contralateral to the stump in each of the patients tested (78.3 ± 4.3% of stimulator output vs 95.0 ± 1.9% of output, $P \leq 0.01$) (fig. 5).

The amplitude of peripheral M responses from biceps and deltoid muscles did not differ significantly in the controls or amputees. Further, in biceps versus deltoid, the controls and amputees showed no significant difference in the side-to-side ratio for the amplitude of MEP and in the side-to-side ratio for percentages of the motoneuron pools activated. We therefore pooled the data from both muscles. The amplitude of M responses

TABLE 2 LATENCIES OF MEPs EVOKED BY STIMULATION OF SCALP POSITIONS LATERAL TO Cz (cm) IN MUSCLES IPSILATERAL AND CONTRALATERAL TO THE STUMP

	MEP latency (ms)						
	Ipsilateral						
Case	*2 cm*	*3 cm*	*4 cm*	*5 cm*	*6 cm*	*7 cm*	*Contralateral*
1				11.5	12.5	12.0	15.0 (n = 1)
				12.5	10.5	14.5	17.0 (n = 1)
				11.5	10.5	13.5	17.0 (n = 1)
3	20.5	13.5	12.5	14.5	14.0		19.5 (n = 1)
	15.0	12.5	13.5	13.5	14.5		18.5 (n = 1)
	16.5	12.5	13.5	14.5	15.0		18.0 (n = 1)
4	12.5	11.5	12.0	13.5			15.0 (n = 1)
	12.5	11.5	12.0	14.0			14.5 (n = 1)
	13.0	12.0	11.5	13.5			15.0 (n = 1)
5	16.0	16.0	17.0				17.5 (n = 2)
	15.0	15.5	16.5				17.0 (n = 2)
	16.5	16.5	17.0				17.5 (n = 2)
6		12.5	12.0	12.5	22.5		12.0 (n = 3)
		11.0	11.5	13.0	20.0		11.0 (n = 3)
			11.5	12.0	18.0		11.5 (n = 3)
7					17.5	18.5	19.5 (n = 1)
					18.0	19.0	17.5 (n = 1)
					17.5	18.0	12.5 (n = 1)
8	13.0	13.0	12.5	13.5	14.5		13.5 (n = 4)
	12.5	13.5	13.0	14.0			13.0 (n = 4)
	11.5	13.0	14.5	12.5			16.0 (n = 4)
			13.5				13.5 (n = 4)
			13.0				13.5 (n = 4)

did not differ significantly between the controls (40 muscles from 10 subjects) and amputees (8 muscles from 8 amputees) (fig. 6). However, the amplitude of MEPs from the muscle ipsilateral to the stump was much larger than that from the muscle contralateral to the stump (0.44 ± 0.10 mV vs 0.07 ± 0.02 mV, $P \leq 0.006$; figs 7, 8A). The side-to-side ratio for the amplitude of the MEPs was larger in the amputees than in the controls (5.95 ± 1.46 in the amputees vs 2.07 ± 0.26 in the controls, $P \leq 0.0006$). When MEP amplitudes were expressed as a percentage of the peripheral M responses, the responses from muscles ipsilateral to the stump were much larger than those contralateral to the stump (16.42 ± 3.28% vs 1.72 ± 0.46%, $P \leq 0.002$) (fig. 8B). The side-to-side ratio for the percentage of the motoneuron pool activated was also much larger in the amputees than in the controls (12.67 ± 4.76 in the amputees vs 2.88 ± 0.60 in the controls, $P < 0.003$).

DISCUSSION

These results show that transcranial magnetic stimuli of equal intensity elicited larger MEPs, and recruited a larger percentage of the motoneuron pool, for muscles immediately proximal and ipsilateral to the stump than for the same muscles on the intact side. Muscles ipsilateral to the stump could be activated from a larger number of scalp positions and at lower intensities of scalp stimulation than those contralateral to the stump. These

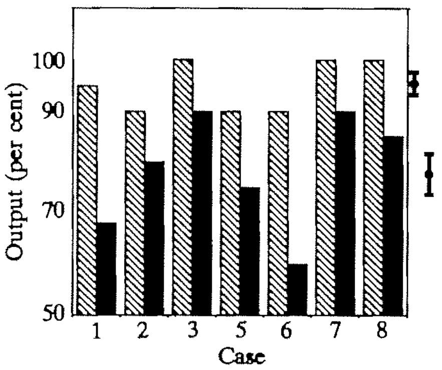

FIG. 5 Threshold for activation of muscles ipsilateral (solid bars) and contralateral (hatched bars) to the stump with stimulation of optimal scalp positions. The ordinate shows the percentage of output of the magnetic stimulator. Means ± SEM are shown on the right of the graph

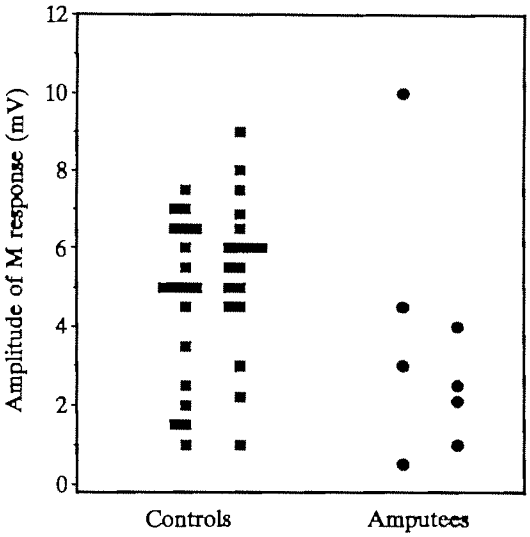

FIG 6. Amplitude of M responses evoked by stimulation of the brachial plexus at Erb's point in a group of control subjects and 8 amputees (*see text*). In each group, two different subgroups are differentiated: responses from the biceps (*left*) and responses from the deltoids (*right*)

findings are consistent with reorganization in adult human motor pathways targeting muscles proximal to the stump after amputation.

The result of a single shock to the motor cortex has been described as a 'powerful excitatory synaptic bombardment of pyramidal tract neurons with facilitation by repeated reexcitation . . .' (Amassian *et al.*, 1987, p. 87). Multiple descending volleys have been recorded after a single anodal electrical stimulus to the motor cortex (Patton and Amassian, 1954). The initial volley, or D wave, is produced by direct excitation of pyramidal tract axons (Kernell and Wu, 1967), while subsequent volleys, or I waves, represent repetitive firing of the same neurons activated transsynaptically by intracortical pathways (Patton and Amassian, 1954; Kernell and Wu, 1967). A single magnetic stimulus seems to activate corticospinal neurons transsynaptically, producing I waves, but not D waves (Day *et al.*, 1987). The depth of stimulation in our studies is limited because the magnitude of the electrical field induced by the magnetic coil drops as a function of the distance to the target neurons, and the coil design optimizes focality

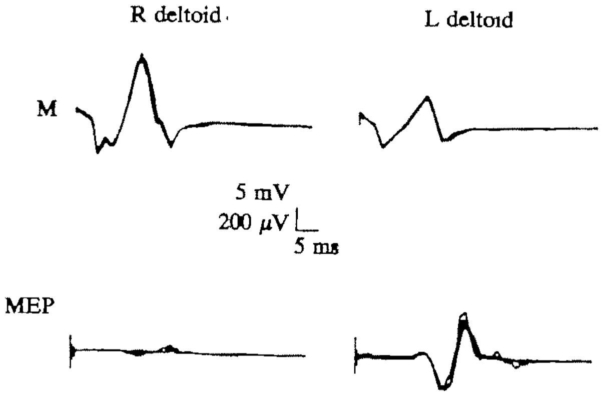

FIG. 7. Peripheral M responses and MEPs from right and left deltoids in *Case 6*, who had an amputation of the left arm above the elbow.

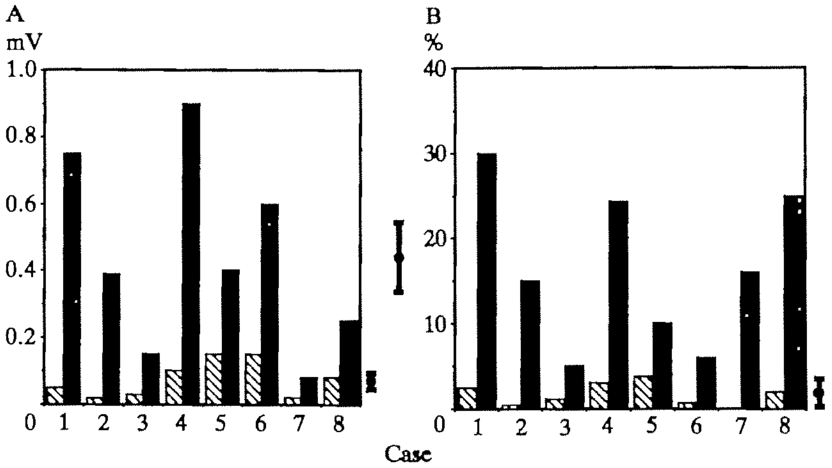

FIG. 8. Amplitude of MEPs for muscles ipsilateral and contralateral to the stump expressed as absolute values (A), and as a percentage of the peripheral M responses (B). Transcranial magnetic stimulation evoked larger MEPs from muscles ipsilateral to the stump (solid bars) than from muscles contralateral to the stump (hatched bars) Means ± SEM are shown on the right of each graph

and sacrifices depth of stimulation (Ueno *et al.*, 1985; Cohen *et al.*, 1990*b*). Consequently, the structures activated are likely to be superficial in the cerebral cortex.

We defined motor representation areas (MRAs) as the number of scalp positions which, on being stimulated with equal intensities, elicited EMG responses from the target muscles. The size of MRAs targeting muscles ipsilateral and immediately proximal to the stump were larger than those for muscles contralateral to the stump and also larger than in the controls (*see* fig. 4). Similar enlargement of MRAs has been reported for proximal arm muscles after quadriplegia (Levy *et al.*, 1990), and for trunk muscles after paraplegia (Topka *et al.*, 1990; Cohen *et al.*, 1990*b*).

The threshold for muscle activation proximal and ipsilateral to the stump was decreased compared with muscles on the intact side. This decrease could partly explain the results of our mapping study. It would also explain the shorter latencies of MEPs ipsilateral to the stump observed in 6 patients. Stimuli of equal intensities would induce larger excitatory postsynaptic potentials in spinal motoneurons and consequently evoke earlier activation of the alpha-motoneuron pool, expressed as MEPs with shorter onset latencies.

Reorganization in motor pathways following deafferentation occurs in rats studied with microstimulation techniques. This work has shown enlarged motor representation areas and decreased cortical thresholds for areas proximal to the stump after neonatal forelimb amputation and after motor nerve injury in adulthood (Donoghue and Sanes, 1988; Sanes *et al.*, 1988). If we assume similar mechanisms in both species (rat and man), these observations support the idea that the size of MRAs and thresholds for muscle activation do change after deafferentation.

Our results do not allow us to determine the level of reorganization (i.e., cortical or subcortical). However, they are compatible with an increased density of physiologically active corticomotoneuronal (CM) connections targeting muscles proximal to the stump. There could be shifts in the targets of CM projections at the spinal level from motoneuron pools corresponding to distal muscles (amputated) or to proximal muscles (preserved). This is possible since single intracortical microstimuli evoke EMG activity from several different muscles (Mitz and Humphrey, 1986), and terminals of a single corticospinal tract axon branch to territories of several motoneuron pools over different spinal segments (Shinoda *et al.*, 1981, 1986). This in turn would lead to an increase in the number of upper motoneurons targeting muscles proximal to the stump per unit of cortical area. An increased excitability of alpha-motoneurons targeting muscles proximal to the stump could also induce similar results.

Two main mechanisms could be responsible for the enlarged map of outputs of muscles immediately proximal and ipsilateral to the stump: (1) unmasking of existing but previously inactive connections, and (2) sprouting with development of new synaptic contacts. Unmasking is a rapid process that may be responsible for short-term plastic changes observed after peripheral nerve manipulations in sensory and motor cortex (Merzenich *et al.*, 1983; Sanes *et al.*, 1988). It is possible that CM connections to distal muscles also project to proximal muscles through higher threshold synapses and, after the amputation, their threshold becomes lowered. Sprouting is a process that requires a longer time. Denervated tissues act as a growth stimulus and new synapses are formed (Raisman and Field, 1973; Matthews *et al.*, 1976). Since our patients had the amputations years before being evaluated, we cannot rule out the participation of any of these mechanisms.

Scalp stimulation induced movement in stump muscles accompanied by a sensation of movement in the missing hand in the 7 patients with acquired amputation, but not in the patient with congenital absence of a hand. Hess *et al.* (1986) reported a similar finding in 1 patient with amputation. Recently, Amassian *et al.* (1989) showed that sensation of movement can occur in normal subjects following a cortical stimulus when the muscles on the contralateral side are paralysed. Under our experimental paradigm, sensation of movement from the missing hand could be produced either by stimulation of preserved representation areas in sensorimotor cortex or by an inappropriate interpretation of afferent signals from the moving stump. In an earlier work, the brain of a patient with congenital absence of a hand showed 'diminution of size of the ascending parietal convolution on the opposite side of the brain occupying precisely the area, stimulation of which, according to the experiments of Ferrier, upon monkeys, causes movements of the opposite hand' (Gowers, 1878, pp. 389–390). Our patient with congenital absence of a limb may never have had a cortical sensorimotor representation area for the missing hand and consequently evidenced no sensation of movement. These findings indicate that at least a part of the cortical representation areas corresponding to the amputated limb maintain a cortical representation for years after the amputation. This conclusion is also supported by the common phenomenon of 'phantom limb'.

Jackson (1931, 1932) suggested that the human motor cortex is organized in terms of 'primary movements'. Since then it has been a matter of debate whether 'muscles' or 'movements' are represented in the motor cortex. For movements to be represented, there should be multiple cortical representation of individual muscles (as suggested by Humphrey, 1986) or at least relatively wide representation of each muscle possibly participating in multiple complex patterns of movement. Different sources of evidence indicate that this is the case (Landgren *et al.*, 1962; Phillips and Porter, 1964; Phillips, 1967, 1969; Jankowska *et al.*, 1975; Kwan *et al.*, 1978*a*, *b*; Sawyer *et al.*, 1979; Strick and Preston, 1982; Mitz and Humphrey, 1986). Our results demonstrate potentiality in adult human motor pathways for reorganization after amputation.

It may be the case that learning of skilled tasks is accompanied by dynamic plastic changes in the map of outputs of the motor cortex targeting muscles involved in the task. For this to occur, there should be a relatively stable set of anatomical topographic relationships over which different maps of outputs could be temporally established by shifting synaptic weights according to the experience of each individual. Our findings support the possibility that this could occur in humans. Further support for this hypothesis comes from studies indicating that adult cortical maps in the somatosensory cortex of primates vary with different and special uses of the hand (Jenkins *et al.*, 1984; Jenkins and Merzenich, 1987).

ACKNOWLEDGEMENTS

We thank Drs G. Goldberg and A. Esquenazi for referring 2 of the patients, Judy Macedo for kindly helping to recruit some of the patients studied, and B. J. Hessie for editorial assistance.

Presented in part at the 19th Annual Meeting of the Society for Neuroscience, Phoenix, Arizona, October 1989.

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(Received October 4, 1989. Revised March 19, 1990. Accepted April 17, 1990)

Brain (1991), **114**, 629–642

MULTIPLE REPRESENTATIONS CONTRIBUTE TO BODY KNOWLEDGE PROCESSING

EVIDENCE FROM A CASE OF AUTOTOPAGNOSIA

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SUMMARY

Body schema disturbances were studied in a 62-yr-old woman with Alzheimer's disease. She was severely impaired in verbal and nonverbal tasks requiring her to localize body parts (on her own body, the examiner's body or a doll's body) even though she correctly named the same parts when pointed at by the examiner. Pointing responses were misdirected mainly to parts contiguous with the target area and, to a lesser extent, to functionally equivalent body parts We also found that the patient was able to define body part names functionally but not spatially In another series of tasks, and in contrast to the above results, performances were normal when small objects, attached to the patient's body, served as pointing targets. Furthermore, on subsequent testing she pointed correctly at the remembered position of these objects. The fact that the same point in 'body space' is localized correctly when it corresponds to an external object and erroneously when it corresponds to a body part contradicts the idea of the body schema as a unitary function. Learning the position of objects on the body surface requires access to some form of body-reference system on which this information can be mapped. We argue that such a system can be available in autotopagnosia and is independent from the visuospatial representations of the body structure that are postulated to be damaged or inaccessible in this syndrome. An integrated account of the present results and of those reported by other authors suggests that multiple levels of representation (e.g., sensorimotor, visuospatial, semantic) are involved in the organization of body knowledge.

INTRODUCTION

Autotopagnosia (Pick, 1922), an incapacity to localize body parts on verbal command (De Renzi, 1982), has classically been considered to reflect an alteration of the mechanisms involved in the generation and maintenance of a spatial representation of the body or body schema. It usually does not occur in isolation as a 'pure' disturbance but is often observed in association with aphasia, apraxia, reaching disorders and unilateral spatial neglect (for reviews *see* Poeck and Orgass, 1971; Denes, 1989).

Despite this association, single case reports indicate that autotopagnosia cannot be reduced to body awareness disturbances (such as hemiasomatognosia) or to a verbal comprehension impairment. De Renzi and Scotti (1970) and Poncet *et al.* (1971) have described patients who were unable to point to body parts (on their own body, a picture or a doll) on verbal command but were capable of naming the same parts when touched

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by the examiner. However, contrary to the classical conception of a disorder specific to the body schema, both De Renzi and Scotti and Poncet *et al.* interpreted the deficit as an incapacity to analyse any whole into its parts, because equally severe difficulties were observed when the patients pointed to different parts of inanimate objects.

The existence of a defect in localizing specific body parts has been demonstrated more recently by Ogden (1985) in a patient who could name body parts, as in the above cases, and who could point to parts of inanimate objects while being unable to point to parts of his own body. Ogden argued that this case supports the idea of a 'discrete body image'. Ogden's case is interesting because it suggests that autotopagnosia is a distinct neuropsychological deficit, body-specific and independent from other impairments of spatial localization. However, it remains unclear what a 'discrete body image' is, and this notion faces the same objections that have been levelled at the body schema concept (Poeck and Orgass, 1971). How is the 'body image' organized and what cognitive operations are necessary to process a representation of the body and/or its parts?

An attempt to clarify these issues has been made by Semenza (1988) who described a case analogous to that of Ogden's. Different verbal and nonverbal body parts localization tasks developed by Semenza and Goodglass (1985) were used. A qualitative analysis of this patient's errors showed that most of her errors consisted of pointing to a body part which shared a functional similarity with the target (i.e., knee for elbow), or to a body part 'contiguous' with the target (i.e., calf for knee). On the basis of these results Semenza proposed that body knowledge is organized 'conceptually'. According to his view, single body parts that are functionally similar are closely related to each other, independently of their actual distance on the body.

However, Semenza did not make clear whether this conceptual organization can be assimilated in the body image or if it is a unitary category of representations. It could be suggested, for instance, that body knowledge may distinguish between nonspatial semantic information such as the functional equivalence between body parts ('joint', 'sense organ', etc.) and other sorts of representations that specify the local and global spatial relationships between body parts. A somewhat similar distinction between semantic and topographic body knowledge has been suggested by Dennis (1976) who described a patient unable to define and understand body part names despite localizing the same body parts in a nonverbal task.

We had the opportunity to study a patient presenting with a striking autotopagnosia in the context of a dementing illness. We used various tasks designed to provide evidence for or against the idea of multiple representations of body knowledge. The results are discussed in terms of a model as to how the body knowledge might be organized.

CASE REPORT

D.L.S. was a 62-yr-old, right-handed housewife with 12 yrs of education. She was referred to the NIH clinical center for the first time in August 1986, because of 'problems with vision' and impaired reading and counting. On the basis of a neurological and neuropsychological evaluation, she received a diagnosis of probable dementia of Alzheimer's type and remained in hospital during her participation in a clinical drug trial. Her cognitive functions became progressively more impaired over the following 3 yrs. We examined her between December 1988 and May 1989. All results (neurological and neuropsychological) reported below refer to this period.

The patient was alert, cooperative, oriented in space but not in time. She complained about difficulty in reading and writing and showed a severe dressing apraxia resulting not from neglect of one side of the body, but from an incapacity to match correctly body parts and parts of clothes. Clinically, she was mildly amnesic in spite of poor performances on standardized tests of memory functioning, since she could recall the names of other patients and medical staff and was able to remember the tests administered during the various sessions. Neurological examination showed normal visual fields. Somatosensory examination was normal; there was no neglect for personal or extrapersonal space; there was no misreaching for objects shown in the foveal or in the peripheral visual field with either hand. A CT scan (fig. 1) in December 1988 showed diffuse cerebral atrophy.

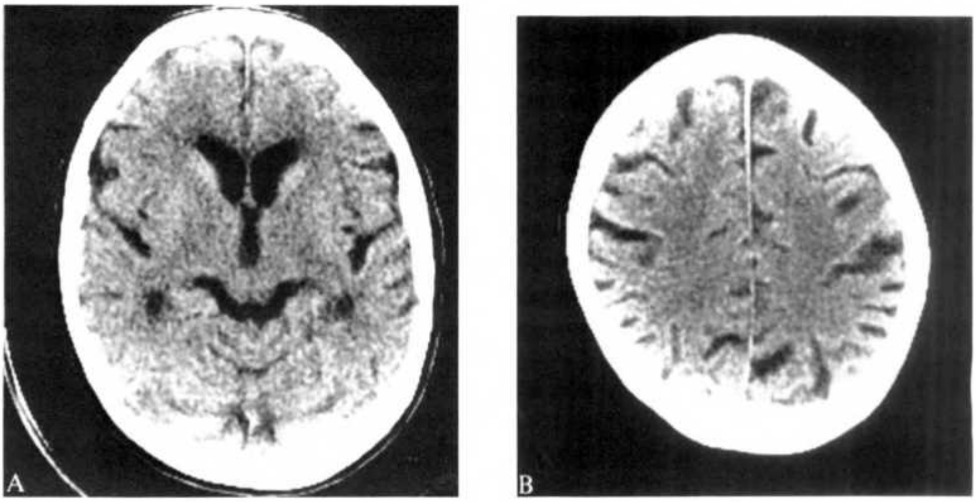

FIG. 1. A, B, CT scan of patient showing diffuse cerebral atrophy.

The general neuropsychological findings are summarized in Appendix I. There were wide-ranging cognitive impairments. Visuospatial abilities were severely affected, in agreement with the major problems of the patient in everyday life as reported by the clinical staff. In addition she presented with a complete Gerstmann syndrome, with agraphia, acalculia, finger agnosia (in both naming and recognition), right-left confusion, and the deficit in which we were most interested, autotopagnosia. The following example is typical of the patient's difficulties with body parts. When she was requested to show where her elbow was, she looked at her body, searching for the elbow and repeating 'yeah elbow, the elbow must be over here', while she was pointing near to the wrist and along the arm. Most of the time, she seemed to be uncertain about the *exact* location of the body part we requested her to point to. The following sections of the paper will focus on our investigation of this disorder.

INVESTIGATIONS

Investigation of body parts

We tested D.L.S.'s ability to localize body parts using verbal and nonverbal instructions.

Verbal instruction. The patient was asked to point to a body part named by the examiner in three different conditions (1) on her own body with the eyes closed/open, (2) on the examiner's body, (3) on a doll's body.

Nonverbal instruction. We asked the patient to point on the examiner's body to the same part that the examiner touched on the patient's body with the eyes closed/open. For example, the examiner touched the patient's shoulder (with the patient's eyes closed) then the patient (with the eyes open) had to touch the examiner's shoulder.

Naming body parts. These were indicated by the examiner on patient's body (with her eyes open/closed) and on the examiner's body as a control task for the comprehension of body part names

General procedure

In our tasks we used 25 body parts (*see* Appendix 2) distributed over the entire body surface, with the exception of the fingers. No requirements were made concerning the side, left or right, of the body part to be localized. We performed several replications of each condition in order to assess the reliability of the patient's performances: *verbal—own body* (n = 5, 4 times with eyes open and once with eyes closed); *verbal—examiner's body* (n = 3); *verbal—doll's body* (n = 3); *nonverbal* (n = 5, 4 times with eyes open and once with eyes closed). Across replications, the patient alternated hands in pointing to the targets. The examiner and patient were always sitting in front of one another. Finally, 10 normal subjects matched for age, sex and education level (mean age and SD: 69.1, 5.2; mean educational level in yrs and SD: 12.9, 1.9), and 2 patients with Alzheimer's disease (both females, aged 75 and 76 yrs, respectively; Global Deterioration Scale Scores of 4, indicating moderate impairment in both cases) were used as controls in all localization tasks except 'verbal-doll's body' and in the naming tasks. One of the Alzheimer patients was not administered the nonverbal version of the pointing task.

Results

The results of the *verbal* conditions (Table 1) showed that the patient's performance was impaired in localizing body parts on herself, on the examiner and on a doll. This deficit was most severe when the patient was required to point to targets located on her own body. Her performance was remarkably constant from one administration to another. The results were not affected by the hand used to respond to whether her eyes were open or closed. Performances of normal and patient controls with Alzheimer's disease were perfect in all conditions tested.

TABLE 1 NUMBER OF ERRORS MADE BY THE PATIENT, 2 PATIENTS WITH ALZHEIMER'S DISEASE (AD) AND 10 NORMAL CONTROLS (NCs) IN LOCALIZING BODY PARTS ON VERBAL AND NONVERBAL COMMAND AND IN NAMING BODY PARTS*

	Patient					*AD1*		*AD2*		*NCs (mean)*	
	I	*II*	*III*	*IV*	*V***	*I*	*II***	*I*	*II***	*I*	*II***
Verbal											
Own body	10	9	10	8							
					10	0	0	0	0	0	0
Examiner's body	6	5	5	–		0		0		0	
Doll's body	5	5	5	–		–		–		–	
Nonverbal	12	10	13	13							
					13	0	0	–	–	0	0
Naming											
Own body	2	2**	–	–	–	1	2	1	1	1	0.6
Examiner's body	2	–	–	–		2		3		0.4	

* Roman numerals refer to the replications of the task (n = 25). ** Tested with eyes closed

In the *nonverbal* modality (Table 1) D.L.S. was also very impaired relative to control subjects. These results were highly reliable and again were not affected by handedness or vision. A comparison of the 5 replications of the verbal and nonverbal conditions showed that significantly more errors were made in the latter than in the former trials ($t(4) = 4.2$, $P < 0.02$). This suggested that the availability of the verbal label for the target may have facilitated its localization.

In contrast to these results, relatively few errors were made in naming body parts indicated by the examiner in either condition. On this task, her performances were similar to those of the 2 Alzheimer's disease controls (Table 1).

Analysis of error types

In order to determine whether the patient's errors were made randomly or whether there existed a relationship between the target and the area to which she pointed, we performed a qualitative analysis of the localization errors for both verbal and nonverbal localization tasks across the different administrations. Three types of errors were scored according to a classification scheme inspired by Semenza and Goodglass (1985): (1) *contiguous*: when the area pointed to is immediately adjacent to the target, i.e., shoulder for upper arm, eyebrow for eye, leg for calf, etc.; (2) *substitution of functionally similar parts*: segments substitution such as upper arm for thigh, forearm for leg, forearm for upper arm, thigh for leg; joint substitutions, such as wrist for ankle, elbow for knee, elbow for wrist, hip for knee, knee for ankle; substitution of eye-ear-nose; (3) *other*: errors that fit in neither of the above categories.

The observed proportions for each of these three error categories must be evaluated against their probability of occurrence by chance, that is, if those errors were produced by random pointing. In order to do so we divided the body surface into 25 nonredundant body parts, only 1 of which could be considered a correct response on a particular trial. In the majority of trials only 2 out of the 24 remaining body parts could fall into the 'contiguous error' category. This implies that random pointing could hit a part contiguous to the target with a probability of about 0.08. The probability is of course smaller for an extremity (only 1 possible contiguous error) and greater for a target located in the middle of the face. The number of possible functional substitutions for limb joints and segments is 5, which means that these errors could occur by chance with about 0.21 probability Since there are only 2 possible substitutions when either eye, ear and nose are targets, the chance probability of this error type for these targets is much smaller than for limbs and joints. Some of the targets had a zero probability for the functional substitution error type (neck, chest). Of course, 'other' errors were the category with the largest probability of chance occurrence. By calculating weighted averages on the entire set of targets for each error category, the following probabilities were obtained: 0.09 for contiguous errors, 0.12 for functional substitutions, and 0.79 for 'other' errors.

Results

In order to facilitate comparisons, only the results for the *verbal-own body* and the *nonverbal* conditions are presented. The distribution of errors of each type is shown in Table 2. It is readily seen that most errors were responses on the area adjacent to the target (contiguous errors): 30 (64%) in the verbal condition and 43 (71%) in the nonverbal condition, proportions much higher than would be expected by chance (7.1:1 and 7.8.1, respectively). In the verbal condition, 'functional substitutions' were more frequent than 'other' errors, with 15 (32%) and 2 (4%) errors respectively. In the nonverbal condition 8 (13%) 'functional substitution' and 10 'other' (16%) errors were found.

TABLE 2 QUALITATIVE ANALYSIS OF PATIENT'S PERFORMANCE IN THE VERBAL (OWN BODY) AND NONVERBAL POINTING TASKS AND ACROSS TRIALS*

	Substitution of functionally similar parts	*Contiguous*	*Other*
Verbal			
I	4	6	0
II	3	6	0
III	2	7	1
IV	3	5	0
V**	3	6	1
Total	15	30	2
Nonverbal			
I	2	8	2
II	0	10	0
III	1	10	2
IV	2	8	3
V**	3	7	3
Total	8	43	10

* No. of errors for each error type. ** Tested with eyes closed

It is clear that in both verbal and nonverbal conditions the proportion of 'other' errors is much lower than would be expected by chance. However, there is one interesting difference between these two experimental conditions: the proportion of 'functional substitution' errors was higher in the verbal than in the nonverbal condition ($\chi^2 = 8.02$, $P < 0.01$). Although the proportion of this error type is greater than expected by chance by a 2.7:1 ratio for the verbal condition, the proportion of errors of this type in the nonverbal condition is close to the chance level (13% observed, 12% expected).

Additional control experiments

Localization of different parts of objects. In order to determine whether the localization problem was specific to body parts or whether it could be interpreted as an 'incapacity to analyse a whole into its parts' (De Renzi and Scotti, 1970; Poncet *et al.*, 1971), we tested the patient's capacity to point to different parts of objects in verbal and in nonverbal conditions. In the verbal condition, the patient had to point to a part of an object named by the examiner. In the nonverbal condition, both patient and examiner held similar objects, but of different sizes. The examiner pointed to a part of the object she was holding and the patient had to point to the same part on the other object. We used 4 small objects in both tasks; in parentheses is indicated the number of parts tested for each object: telephone (n = 5), truck (n = 9), table (n = 3 verbal, n = 4 nonverbal), blouse (n = 5).

D.L.S's performance on this task was near perfect; only 1 error occurred in the verbal condition when pointing to a part of a telephone. Thus her deficit cannot be explained by a general problem in identifying part/whole relationships.

Body part names: spatial vs functional definition. We were also interested to know if D.L.S. was able to define *spatially* and *functionally* body parts that she could not localize. This might provide some information on the status of the patient's conceptual knowledge about body parts in a situation that does not require an explicit localization response.

The examiner stood behind the patient to minimize visual cues, and asked her to define verbally the location of 6 body parts (ear, nose, mouth, eyes, knee, wrist). The questions were formulated in this way: where are the ears located?, where is the wrist located? If no answer was obtained, we used a two-choice forced recognition probe task administered in the following way: 'Are the ears next to the mouth?' or 'Is the wrist next to the forearm?' D.L.S. failed completely on this task, despite being allowed to inspect her own body visually throughout the task. Not only could she not describe body parts locations verbally, but she performed at chance on the recognition probe task.

We then asked her to define the functions of 6 body parts (ear, nose, mouth, eyes, hands, arms). The questions were posed in the following manner: 'What is the mouth for?' or 'What are the hands for?', etc. She answered quickly and adequately to all questions.

Localization of objects on the body

The results presented thus far seem to indicate the presence of specific difficulties in mapping identified body parts on a global map of the body. This raises the question as to whether *any* spatial information can be coded using a body map as a reference system. Ogden's (1985) informal observation that her autotopagnosia patient remained able to point to different parts of clothes he was wearing speaks to this issue. In order to address this question systematically, we submitted the patient to a task which used her body as a reference for the localization of *objects* attached to her body and not for the localization of the body parts themselves.

Methods

A subset of 10 body parts was taken from the previous tasks and the same general procedure was followed but with a variation: the target could be either a body part or a small object attached on the patient's body (fig. 2). The following pairs were made: car-right forearm, bed-left shoulder, soldier-right upper arm, square-left elbow, earring-left wrist, rubber band-left hand, figurine-left knee, paper sticker-left calf, chair-chest, toothbrush-right thigh.

D.L.S. was requested to point to a body part or to an object attached on the same location. Two types of instructions, verbal and nonverbal, were used The patient either had to point to the object *named* by the examiner (verbal), or was requested to point on the examiner's body to the same object that had been

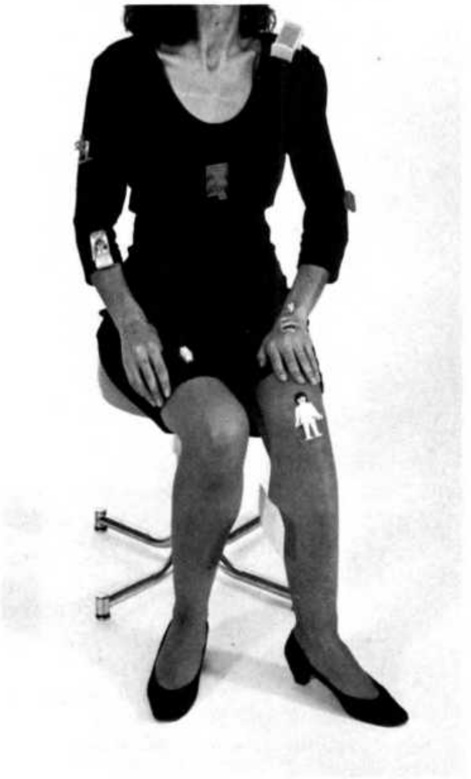

FIG. 2. Picture showing arrangement of targets on the patient's body in the object pointing task (*see* text for details).

touched by the examiner on the patient's body (nonverbal; note that both examiner and patient had identical objects attached on the same parts of their bodies). This was contrasted with the same tasks performed with body parts as targets without any object being present on anyone's body. Several replications of each condition were obtained in order to assess the patient's reliability.

Results

The results shown in Table 3 are consistent with the previous results in that a large number of errors were made when body parts were used as targets. However, when the targets were objects attached to the same body parts, the patient's performance was virtually perfect.

Pointing from memory to the position of the objects

It can be argued that the patient's success in this task was the result of an efficient visual search strategy. In order to determine whether this was the explanation, or whether she could actually locate the objects purely on the basis of knowledge of their position on the body surface, the patient was subsequently asked to point to the remembered location of these objects in their absence.

Methods

We performed 4 replications of this task. A delay of 24 h separated the task with objects attached to the patient's body and the first administration of this location memory task. A delay of 14 days separated the first administration of this memory task from the last, with intervening tests at 2 and 7 days. During that period the objects were never attached to the patient's body, so there was no possibility of relearning during this interval.

TABLE 3 NUMBER OF ERRORS MADE BY THE PATIENT IN POINTING ON VERBAL AND NONVERBAL COMMAND TO BODY PARTS AND TO OBJECTS ATTACHED ON THE SAME BODY PARTS*

Verbal	*I*	*II*	*III*	*IV*	*V*	
Body parts	6	5	6	6	6	
Objects on body parts	1	1	1	0	0	
Nonverbal	*I*	*II*	*III*	*IV*	*V*	*VI*
Body parts	5	5	6	5	7	6
Objects on body parts	0	1	0	0	0	0

* Roman numerals refer to the replications of the task (n = 10).

Results

D.L.S.'s performance was perfectly accurate and remained so up to the 15 day retest. She always pointed quickly and without hesitation to the remembered positions of the objects. She did not make right-left confusions for targets not located close to the body midline. Interestingly, she never named, spontaneously or on request, the body parts objects were attached over, suggesting that she was not using a verbal strategy (e.g., the names of the body parts) in the retrieval of the objects' position.

Thus the results of this task show that the patient had coded in long-term memory the position of the objects onto a map of the body surface.

Localization of body parts in the presence of the objects

In the final task the capacity of the patient to localize the same 10 body parts was reassessed while the objects were still attached to her body. This was done in order to test the possibility that she might improve her performance by using the objects as landmarks for the target body parts.

Methods

We once more attached the objects to the patient's clothing, but gave her verbal instructions, stressing that this time she was to point to different body parts on command and ignore the objects. We administered this task 3 times on separate days.

Results

D.L.S.'s performance on this task indicated that the presence of objects did not facilitate the localization of the corresponding body parts and this finding was confirmed for all replications of the task. She made 6/10 errors for the first replication and 9/10 for the second and third.

DISCUSSION

Our study suggests that the breakdown of what has classically been called the 'body schema' may occur at particular stages or levels of body knowledge processing and supports the idea of a body image composed of multiple representations. Specifically, our results demonstrated that D.L.S. could not localize body parts on herself or on another person either on verbal or nonverbal command, but that she could name body parts. She could not define body part names spatially but she could define body part names functionally. The patient made two main types of localization error: contiguity errors and functional substitutions. Contiguity errors were the most frequent across all task conditions. In the verbal task the proportion of functional substitutions was higher than in the nonverbal task, and in fact this error type occurred above chance level only in the verbal condition. A striking finding was that the patient could point to objects attached to different parts of her body even though she could not localize those body parts themselves. In addition, she could reliably point to the remembered position of all these objects. Nevertheless, the presence of objects did not improve the patient's performance when she was instructed verbally to point to the body parts where the objects were currently attached.

It must be stressed that the patient's localization deficit was specific to body parts. Her preserved ability to point to different parts of inanimate objects means that an interpretation of her deficit as a general incapacity to analyse *any* whole into its component parts is inadequate (De Renzi and Scotti, 1970; Poncet *et al.*, 1971). It can be argued that a human body is much more complex than other objects. Comparability of performances in pointing to parts of a body and of inanimate objects may also be limited because fewer parts were sampled in the latter than the former tasks. However, although our patient and the patients reported by De Renzi and Scotti (1970) and Poncet *et al.* (1971) had quantitatively similar impairments in localizing body parts, our patient, unlike theirs, did not show impaired localization of parts of objects. A verbal comprehension deficit for body part names or for task instructions cannot play a role in our interpretation of the results for several reasons: the patient could name body parts and could define, at least functionally, body part names; her performance was consistently worse in the nonverbal than in the verbal task, and she understood the instructions of the localization tasks insofar as she pointed correctly to parts of objects and to objects attached to her body. Thus our results confirm and extend those previously reported by Ogden (1985) and more recently by Semenza (1988), in that autotopagnosia can exist as a localization deficit *specific* to body parts.

A model of 'body knowledge' representation

The present study was an attempt to understand further the types of representations and processing necessary to perform localization of body parts tasks. Our results lead us to propose a general framework, shown in fig. 3, which attempts to delineate both the nature and levels of representations from which an 'image' of an individual's own body may emerge. We hypothesize that at least four kinds of representations contribute to body knowledge processing.

1. The first contains semantic and lexical information about body parts, such as names, the functional relations that exit between body parts, such as the wrist and the ankle (e.g., articulations), the functional purpose of the mouth or the ear, etc. These representations are in large part propositional and are likely to be more strongly linked to the verbal systems.

2. The second contains the category-specific visuospatial representations of an individual's own body but also of bodies in general. These representations define a structural description of the body and specify in a detailed manner the position of individual parts on the body surface (e.g., the nose is in the middle of the face), the proximity relationships that exist *between* body parts (e.g., the nose is next to the eyes; the leg is between the ankle and the knee, etc.) and most importantly the *boundaries* that define each body part. These representations are necessary for 'part/whole' analysis.

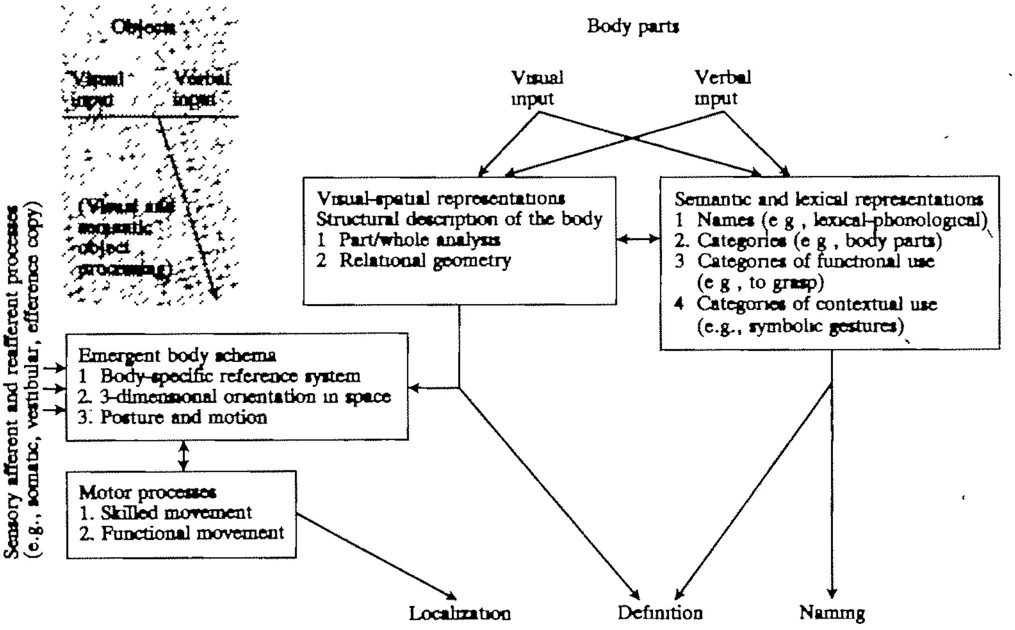
Objects
Visual input
Verbal input
(Visual and semantic object processing)
Body parts
Visual input
Verbal input
Visual-spatial representations
Structural description of the body
1 Part/whole analysis
2 Relational geometry
Semantic and lexical representations
1 Names (e g , lexical-phonological)
2. Categories (e g , body parts)
3 Categories of functional use (e g , to grasp)
4 Categories of contextual use (e.g., symbolic gestures)
Sensory afferent and reafferent processes (e.g., somatic, vestibular, efference copy)
Emergent body schema
1 Body-specific reference system
2. 3-dimensional orientation in space
3. Posture and motion
Motor processes
1. Skilled movement
2. Functional movement
Localization
Definition
Naming

FIG. 3. Schematic diagram of postulated representations contributing to processing of body knowledge. Dashed box indicates postulated locus of the functional impairment in autotopagnosia. The shaded area on the left is added in order to account for the results in the objects pointing task.

They are likely to be more strongly linked to the nonverbal visual and somatic sensory systems.

3. The third level is the emergent body-reference system and is conceptualized as a dynamic, actual body image. It gives information about the position and the changes in position of an individual's own body parts relative to each other and in relation to the external space. It is a polymorphous 'system' that emerges from various sources of sensory information: the somatosensory homunculus, as well as vestibular and visual afferences.

4. Motor representations also contribute to the construction of a spatial representation of the body. Theories of sensorimotor organization typically assume that movements in space are coded with respect to some form of body-centred reference and in turn that movement execution generates information (by way of corollary discharge and/or polysensory reafference) for the coordination of body and external space representations (Paillard, 1971). It therefore appears reasonable to suggest that representations of motor programs contribute to the construction and maintenance of an emergent body-reference system as defined above, but also at a more symbolic level, to the establishment of categorical knowledge of functional and contextual use of body parts.

The components of a body representation system are hypothesized to be relatively independent but can also interact with one another. The degree of involvement of each component in body parts tasks may depend on particular task demands (fig. 3). For instance, naming or defining functionally a body part probably requires mostly the participation of the semantic and lexical representations. The localization of a specific body part, whether it is expressed by a pointing gesture or a verbal response, is postulated to be mediated principally by the visuospatial representations. The emergent body-reference system is a necessary step in body parts localization since only it can provide information about the *actual* location of the body segments.

The idea that body knowledge processing might involve different types of representations is not new. A 'body image' linked to the verbal (Selecki and Herron, 1965) or semantic systems (Dennis, 1976) has already been hypothesized. The 'conceptual knowledge' of the body evoked by Semenza (1988) probably refers to the same processes. The existence of a visual mental image in which the relations between body parts are defined has also been alluded to by Ogden (1985). Finally, the third level described here is very close to the postural schema hypothesized by Head and Holmes (1911). To our knowledge, however, the model we propose is the first synthetic description of body knowledge representations and we believe that it can account for both our results and those reported in other patients with autotopagnosia.

We propose that the deficit observed in our patient involves mostly the visuospatial representations while the semantic representations and the body-reference system appear intact.

Spatial and semantic body representations: two distinct types of knowledge

Several aspects of the present results support the distinction between these two categories of representations. First, there is the independence between naming and localization of body parts. Our patient, as for most patients with autotopagnosia, could name body parts that she could not localize. Interestingly, the reverse pattern of results was reported by Dennis (1976) in a patient who could localize body parts (with nonverbal instructions) but could not define or understand names of body parts. This suggests a double dissociation of the processing systems involved in these two tasks. Secondly, there is the dissociation between definition of functional (preserved) and spatial (impaired) attributes of body part names shown by our patient. Both Ogden (1985) and Semenza (1988) have reported similar results using analogous tasks. Thirdly, there is the prevalence of 'contiguity' errors in the localization of body parts in our patient, which suggests a 'fuzzy' knowledge of proximity relations and of boundaries between body parts. This type of error was much more frequent than functional substitutions which can be considered a more 'semantic' class of error.

All our findings point to a marked impairment of spatial knowledge while lexical and semantic knowledge about body parts is preserved (fig. 3). The overall greater impairment in nonverbal tasks suggests that spatial representations are normally activated mainly by visual inputs. The larger number of 'functional substitution' errors in the verbal tasks probably reflect the coactivation of semantic information. This activation presumably cannot provide a *location* for the target, but may contribute to reducing the range of choices to a set of functionally equivalent body parts. Alternatively it could be argued that the patient's deficit reflects a partial breakdown in lexical/semantic representation and that functional substitutions reflect a genuine semantic confusion. However, this is contradicted by the fact that such semantic confusions did not occur when she only had to name, or to define functionally, the same body parts. Thus her impairment is more likely to be located at a later stage, either in the visuospatial representations themselves or in accessing these representations (fig. 3).

Direct access to a body reference system

Evidence for a direct access to a body-reference system comes from the results on the object position memory task. The patient's performance on this task is a strong argument for an intact body-reference system that can be used to code object positions and retrieve them from long-term memory (fig. 3). It indicates that this system can be accessed directly in a task that does not require a prior analysis of the semantic and spatial characteristics of the body parts on which objects were placed.

We therefore propose that autotopagnosia is an impairment which can affect predominantly, if not exclusively, visuospatial representations relative to the body structure. An important implication of these results is that such visuospatial knowledge may be distinct from, and independent of, the semantic representations of the body and the body-reference system.

ACKNOWLEDGEMENTS

We wish to express our gratitude to Ms K. Putnam for supplying part of the patient's clinical neuropsychological assessment. Special thanks are due to D L.S. for her kind and patient cooperation throughout the many hours of testing. We thank the National Institute of Neurological Disorders and Stroke, and the Medical Neurology Branch, for providing facilities and support. The opinions and assertions contained herein are the private views of the authors, and are not to be construed as official or necessarily reflecting the views of the National Institutes of Health, the United States Public Health Services, or the Uniformed Services University of the Health Sciences.

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(*Received December 19, 1989. Revised March 30, 1990. Accepted April 17, 1990*)

APPENDIX 1

Patient's general neuropsychological assessment

Global Deterioration Scale	4 (moderate)	
MATTIS Dementia scale		
Global score	108 (max = 144)	
Attention	30 (max = 37)	
Initiation and perseveration	25 (max = 37)	
Construction	2 (max = 6)	
Conceptualization	31 (max = 39)	
Memory	20 (max = 25)	
Memory		
Wechsler Memory Scale		
Information	3	
Orientation	4	
Mental Control	3	
Memory Passages	1	
Digits Total	5	
Visual Reproduction	0	
Associate Learning	1 5	
MQ	59	
Buschke Selective Reminding Test	Patient	Controls (n = 37)
Recall	4.50	8 37 (1.38 ST)
Consistency	0.24	0.67 (0.18 ST)
Language		
Verbal Fluency (90 s)	Patient	Controls
Word Association (health)	6.5	19 (6 77 STD) (n = 40)
Letter (S)	4	23 (5.82 STD) (n = 40)
Category (animals)	7	25 (6.29 STD) (n = 19)
Sentence Completion	41/42 (correct)	
Reading	Impaired	
Writing	Impaired	
Visual perception		
Naming (pictures)	14/15 (correct)	
Drawing	Severely impaired	
Object assembly	Unable to perform	
Block design	Unable to perform	
Stereognosia		
Naming		
Left hand	15/15 (correct)	
Right hand	15/15 (correct)	
Apraxia		
Ideomotor	Normal	
Ideational	Impaired	

APPENDIX 2

Body parts stimuli used in the verbal and nonverbal localization tasks and in the Naming task

1. Nose
2. Wrist
3. Knee
4 Hand
5. Shoulder
6 Lips
7. Eye
8. Hair
9. Foot
10. Calf
11. Forearm
12 Chest
13. Neck
14 Ear
15. Thigh
16. Waist
17. Eyebrow
18 Hips
19 Chin
20 Toe
21 Cheek
22 Forehead
23 Upper arm
24. Ankle
25. Elbow

APPENDIX 3

Number of instances (in brackets) in which the pointing responses were directed to body parts other than the target, across all replications of the verbal *(own body) and* nonverbal *tasks. Error type: C = contiguous, FS = functional substitutions, O = other.*

Verbal

Target	*Response*
Wrist	Elbow (4 FS), ankle (1 FS)
Hand	Forearm (3 C), knee (2 O)
Shoulder	Upper arm (1 C), neck (2 C)
Lips	Chin (1 C)
Eye	Forehead (2 C)
Calf	Thigh (4 C), leg (1 C)
Forearm	Elbow (1 C), thigh (4 FS)
Ear	Cheek (1 C)
Thigh	Hip (5 C)
Eyebrow	Forehead (1 C)
Hip	Waist (3 C)
Upper arm	Shoulder (4 C), elbow (1 C)
Ankle	Wrist (1 FS)
Elbow	Ankle (2 FS), wrist (2 FS), knee (1 FS)

Nonverbal

Target	*Response*
Wrist	Forearm (1C), elbow (2 FS), ankle (1 FS), neck (1 O)
Knee	Thigh (3 C), waist (1 O)
Hand	Wrist (1 C), nose (1 O), chest (1 O), shoulder (1 O)
Shoulder	Upper arm (2 C)
Lips	Chin (2 C)
Eye	Cheek (1 C), eyebrow (2 C), ear (1 FS)
Hair	Forehead (1 C)
Foot	Ankle (1 C)
Calf	Leg (3 C), forehead (1 O)
Forearm	Elbow (2 C), upper arm (1 FS), chin (1 O)
Neck	Chin (3 C), forehead (1 O)
Ear	Cheek (1 C), eye (1 FS)
Thigh	Hip (1 C), hand (1 O)
Waist	Chest (2 C)
Eyebrow	Forehead (1 C), eye (1 C)
Hips	Waist (3 C)
Chin	Neck (2 C), cheek (1 C)
Cheek	Neck (2 C), chin (1 C)
Upper arm	Shoulder (1 C), (cheek O)
Ankle	Foot (1 C), elbow (1 FS)
Elbow	Upper arm (3 C), forearm (1 C), wrist (1 FS)

Brain (1991), **114**, 643–662

RESPONSES OF SINGLE SPINAL MOTONEURONS TO MAGNETIC BRAIN STIMULATION IN HEALTHY SUBJECTS AND PATIENTS WITH MULTIPLE SCLEROSIS

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SUMMARY

Magnetic stimuli delivered over the scalp can cause single motor units to discharge in intrisic hand muscles. The discharge characteristics of 26 tonically active, low threshold single motor units in the first dorsal interosseous muscle of 13 healthy subjects and of 21 motor units in 9 patients with multiple sclerosis (MS) were studied. Up to 500 transcranial magnetic stimuli were delivered at the vertex and were given randomly with respect to, or at a fixed delay after, the previous voluntary discharge. Peristimulus time histograms (PSTHs) of motor unit discharges were constructed. In healthy subjects, two periods of increased firing probability were seen at onset latencies of 20–31 ms and 56–90 ms after the stimulus. These periods have been termed the primary peak and the secondary peak. The primary peak had a mean duration of 4.6 ms and was found to be multimodal in 17 motor units. Subpeaks had intermodal intervals of between 0.6 and 2.4 ms. Subpeaks probably result from a sequence of excitatory postsynaptic potentials (EPSPs) induced at the motoneuron by corticospinal impulses.

In patients with MS, the primary peak could be absent, delayed in onset, of increased duration without discernible subpeaks, or showing increased intermodal intervals between subpeaks. In 3 motor units from patients with MS, PSTHs with normal features were found. It is postulated from this study of the corticospinal inputs to single motoneurons that motor impairment in MS can be due to a number of mechanisms including slowed conduction in corticospinal fibres, dispersion of arrival times of corticospinal impulses at spinal motoneurons and conduction block in corticospinal fibres, which may be frequency dependent.

INTRODUCTION

This study has examined the firing characteristics of single motor units in the first dorsal interosseous muscle (FDI) of the hand in response to magnetic brain stimulation in both healthy subjects and patients with multiple sclerosis (MS). The aim was to increase understanding of the neural mechanisms of motor impairment in MS.

In baboons, single anodal electrical stimuli applied to the hand area of the motor cortex cause a train of impulses to descend in the corticospinal tract to the spinal motoneurons serving intrinsic hand muscles (Hern *et al.*, 1962; Landgren *et al.*, 1962; Kernell and Wu, 1967*b*). Recordings from the pyramidal tract show an initial direct (D) wave due to activation of corticospinal cells followed by a variable number of indirect (I) waves (Patton and Amassian, 1954). Impulses in the D and I volleys travel over the same corticospinal fibres (Kernell and Wu, 1967*a*). Such D and I waves have also been recorded from the human spinal cord in response to electrical scalp stimuli (Boyd *et al.*, 1986;

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Katayama *et al.*, 1988). Intracellular recordings from primate motoneurons show that the descending impulse train produces a series of excitatory postsynaptic potentials (EPSPs) often accompanied by an inhibitory postsynaptic potential (Landgren *et al.*, 1962; Phillips and Porter, 1964; Kernell and Wu, 1967*b*). The initial excitatory effects are transmitted monosynaptically from corticospinal axons (Bernhard and Bohm, 1954; Preston and Whitlock, 1961; Landgren *et al.*, 1962), whereas the inhibitory effect is thought to be transmitted via the Ia inhibitory interneuron (Jankowska *et al.*, 1976). Temporal summation of these EPSPs may be sufficient to bring the motoneuron membrane potential to firing threshold. There is also convergence of many motor cortical axons on to single spinal motoneurons (Phillips, 1986) and spatial summation of excitation may also be important in causing the motoneuron to fire. It has also been shown (Muir and Porter, 1973; Fetz and Cheney, 1980) that corticospinal synapses on motoneurons exhibit facilitation such that the EPSPs produced by successive impulses are larger than expected from simple linear summation. Direct approaches to obtain comparable information on the human motor system are not possible and less invasive techniques must be used.

The discovery that electrical (Merton and Morton, 1980) and magnetic stimuli (Barker *et al.*, 1985) applied to the scalp of awake and cooperative humans could excite the motor cortex, and that such stimuli if sufficiently weak were capable of activating single motor units (Hess and Mills, 1986; Zidar *et al.*, 1987) has allowed fundamental information on the human corticospinal connection to be obtained. Magnetic stimuli have been shown to produce multiple peaks of increased short latency firing probability in peristimulus time histograms (PSTHs) from single intrinsic hand muscle motor units, suggesting the arrival at the motoneurons of a sequence of descending impulses (Mills, 1988). Longer latency effects also occur following electrical or magnetic transcranial stimulation (Calancie *et al.*, 1987; Colebatch *et al.*, 1989). Magnetic stimulation differs from electrical in a number of respects: the latency of surface recorded responses in intrinsic hand muscles is some 2 ms longer (Hess *et al.*, 1986), and the facilitatory effects of background voluntary contraction are more pronounced with magnetic stimulation (Hess *et al.*, 1987*a*). It is thought that electrical scalp stimuli probably initially excite motor cortical cells or their axons directly, whereas magnetic stimuli, at least when applied with the coil tangential to the scalp and with the inducing current travelling clockwise when viewed from above, probably excite motor cortical cells transynaptically via horizontally oriented neurons (Day *et al.*, 1987, 1989). Hand muscle motor units activated by transcranial stimuli are always among the first recruited by minimal voluntary activation and it seems likely that, irrespective of whether the descending motor command is generated voluntarily or by an external stimulus, the motoneuron size principle (Henneman, 1957) in the cord selects which motoneuron will fire.

The present experiments have examined the firing probabilities of single motor units in the FDI muscle with respect to transcranial magnetic stimulation of the motor cortex. Results in healthy subjects indicate that part of the human corticospinal connection is probably monosynaptic and that single stimuli to the motor cortex cause repetitive high-frequency firing of cortical cells. In patients with MS, a number of abnormalities have been discovered which may explain at the synaptic level some aspects of their motor impairment. Abbreviated reports of some of this work have already been published (Boniface and Mills, 1989; Boniface *et al.*, 1989*a*, *b*).

METHODS

The discharge characteristics of 26 tonically active, low threshold motor units in the right FDI muscle of 13 healthy subjects ranging in age from 22 to 46 yrs and 21 motor units in the FDI of 9 patients with MS ranging in age from 35 to 58 yrs were studied. All subjects and patients were right-handed and gave their informed consent to the experiments which were performed with the approval of the local ethics committee. Patients were classified according to the criteria proposed by Poser *et al.* (1983). Clinical details of the patients are given in Table 1. Impaired fine finger movement was present in all patients except Case 1 but its interpretation was complicated by the presence of other neurological deficits affecting the hand.

Magnetic stimulation

The characteristics of the magnetic stimulator (Novametrix Inc.) have been given previously (Hess *et al.*, 1987*a*). The 26 turn flat circular copper coil, outside diameter 13 cm, was held in place tangentially to the scalp with a helmet with its centre over the vertex. Although the scalp position is not critical and the same single motor unit can be caused to fire from widely differing scalp locations (Hess *et al.*, 1987*b*), it was found that the lowest intensities were effective at the vertex. As weak stimuli were used, it was important to maintain the same coil position and orientation throughout the experiment. The inducing current when viewed from above flowed clockwise or anticlockwise for recordings from the left or right FDI, respectively. Recordings were made from the right FDI in all healthy subjects. In each experimental session, 100–500 stimuli were given. The rate of stimulation never exceeded 1 per 3.5 s.

The output of the stimulator is indicated as a percentage of its maximum output; the voltage induced in a search coil at a fixed position relative to the stimulating coil has been shown to be a linear function of nominal output (Hess *et al.*, 1987*a*). The nominal stimulus intensities used in healthy subjects fell in the range from 35 to 53% of the maximal output of the stimulator. In patients, stimulus intensities of 50 to 73% were used. At the start of each experiment, a short series of trials was undertaken to select a stimulus intensity high enough to cause the motor unit to discharge at the primary peak latency (*see below*) but not so high as to produce many compound discharges.

Two stimulation protocols were used (fig. 1). In the first, stimuli were delivered regularly every 3.5 s. Stimuli fell randomly in the ongoing motor unit spike train; this was confirmed by examining the distribution of the intervals between the stimulus and the previous voluntary spike. In the second, a motor unit spike was used to trigger the stimulator after a fixed delay of 10–80 ms. The former are referred to as random stimulation experiments, the latter as spike-triggered stimulation experiments. Only the random stimulation protocol was used in patients.

Recording

The subject was seated with the arm resting in a comfortable position supported on a horizontal platform. Isometric contractions were made against resistance. Subjects were asked to maintain repetitive motor unit firing and were aided by auditory and visual feedback. The mean (±SD) interspike interval (ISI) for all motor units taken together was 115 (±22) ms for healthy subjects and 112 (±19) ms for MS patients. Single motor unit potentials were recorded using fine concentric needle electrodes (Dantec, type 13L58). Signals were amplified with a band pass of 32 Hz–16 or 32 kHz (Medelec, type MS6) and epochs of −250 to +250 ms relative to the stimulus were digitized at 10 kHz (Cambridge Electronic Design, 1401) for subsequent analysis. In some early experiments on healthy subjects, a prestimulus epoch of 50 ms was collected. In spike-triggered experiments, motor unit spikes were passed to a window discriminator (World Precision Instruments, type 121) and output pulses were used to trigger the magnetic stimulator after a fixed delay. Epochs of −250 to +250 ms relative to the triggering spike were collected.

Data analysis

PSTHs were constructed off-line, which had several advantages: on some occasions motor units other than the one of interest were fired by the stimulus, making accurate discrimination of the index spike difficult (fig. 1D); the artefact produced by the stimulus did not interfere with measurement of motor unit discharges in its proximity; and by recording each trial individually, it was possible to sort trials into those in which a spike occurred in the primary peak (PP) trials from those in which it did not (*see* fig. 5). The few trials in which the index motor unit potential could not be discriminated with certainty were discarded. The time of occurrence to the nearest 0.1 ms of individual motor unit spike discharges was measured by a

TABLE 1 CLINICAL DETAILS OF PATIENTS

Case	*Sex*	*Side studied*	*Poser classification**	*Finger/nose ataxia*	*Tone increased at elbow or wrist*	*Exaggerated finger jerks*	*Upper limb reflexes*	*FDI strength (MRC)*	*Sensory abnormality*
1	M	L	CPMS C1	+	–	–	E	4	–
2	F	R	CDMS A1	+	–	+	E	4	$+^1$
3	M	L	CDMS A1	**	+	–	N	2	–
		R		**	–	–	N	4	–
4	M	R	LSDMS B2	+	–	–	N	5	–
5	F	L	CDMS A1	–	–	+	E	4	$+^2$
		R		–	–	+	E	4	–
6	M	L	CDMS A2	–	–	–	N	4	$+^3$
7	F	R	CDMS A1	–	–	–	N	5	$+^4$
8	F	R	LSDMS B3	–	–	–	N	4	$+^2$
9	F	R	CDMS A1	–	–	–	N	4	$+^2$

* CDMS = clinically definite MS· CPMS = clinically probable MS: LSDMS = laboratory supported definite MS. ** Proximal weakness and/or increased tone precluded testing. + = present. – = absent. E = exaggerated. N = normal. [1] = impaired light touch, vibration and joint position sense; [2] = impaired light touch; [3] = hyperaesthesia; [4] = impaired vibration sense

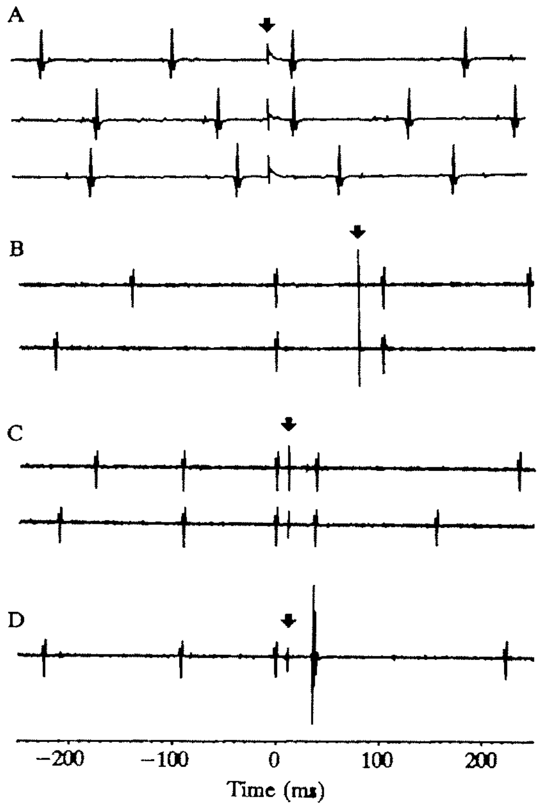

FIG. 1. Recordings from single FDI motor units from a healthy subject. The motor units were being driven voluntarily at 8–12 discharges/s. Magnetic stimuli were delivered at times indicated by arrows. A, random stimulation protocol in which the stimuli fell randomly within the ongoing motor unit spike train. In the *upper* 2 records the motor unit fired at the PP latency in the lower record at the SP latency. B, spike-triggered stimulation protocol in which a voluntarily occurring spike at time zero triggered the stimulator after a delay of 80 ms. The motor unit fired at the PP latency. C, spike triggered trials in which the same motor unit as B triggered a stimulus at a delay of 10 ms. The motor unit again fired at the PP latency. D, the spike-triggered stimulus caused a compound motor unit discharge; thus trial was excluded from the analysis.

cursor on the computer display. PSTHs and cumulative sums were constructed with bin widths of 1 ms and 0.2 ms (*see* figs 2A, 3). The mean discharge frequency calculated from the period before the stimulus was used as a basis for defining periods of increased firing probability after the stimulus thus taking into account both the prestimulus firing rate and the number of trials. Definitions of the major peaks and subpeaks in PSTHs are given in fig. 2. In some patients there was an apparent lack of an excitatory response which was confirmed using the cusum technique (Davey *et al.*, 1986).

RESULTS

Healthy subjects

Major peaks in PSTHs. All motor units studied showed an increase in firing probability beginning at between 20 and 31 ms after the stimulus, termed the primary peak (PP). There was then a pause in motor unit discharge followed in 10 motor units by a second rise in firing probability, the secondary peak (SP) with an onset latency of between 56 and 90 ms after the stimulus (fig. 2D). In the 16 motor units without an SP there was almost always a rise in firing probability with a peak at around 70 ms, but the peak did not fulfil the criteria set out in fig. 2D. PP had a mean (±SD) duration of 4.6 (±1.7) ms with a range of from 2 to 9 ms. In contrast, SP had a mean (±SD) duration of 20.2 (±9.3) ms with a range from 5 to 39 ms. The height of SP was always smaller than that of PP. SP was never found in isolation. PP was always abruptly terminated by a period of zero firing probability and was always unimodal when examined with 1 ms bin widths.

Subpeaks within the primary peak. When plotted with a time resolution of 0.2 ms, PP in 17 out of 26 motor units was seen to consist of 2 or 3 subpeaks (figs 2C, 3B). The mean (±SD) intermodal interval between subpeaks taking all the data together was

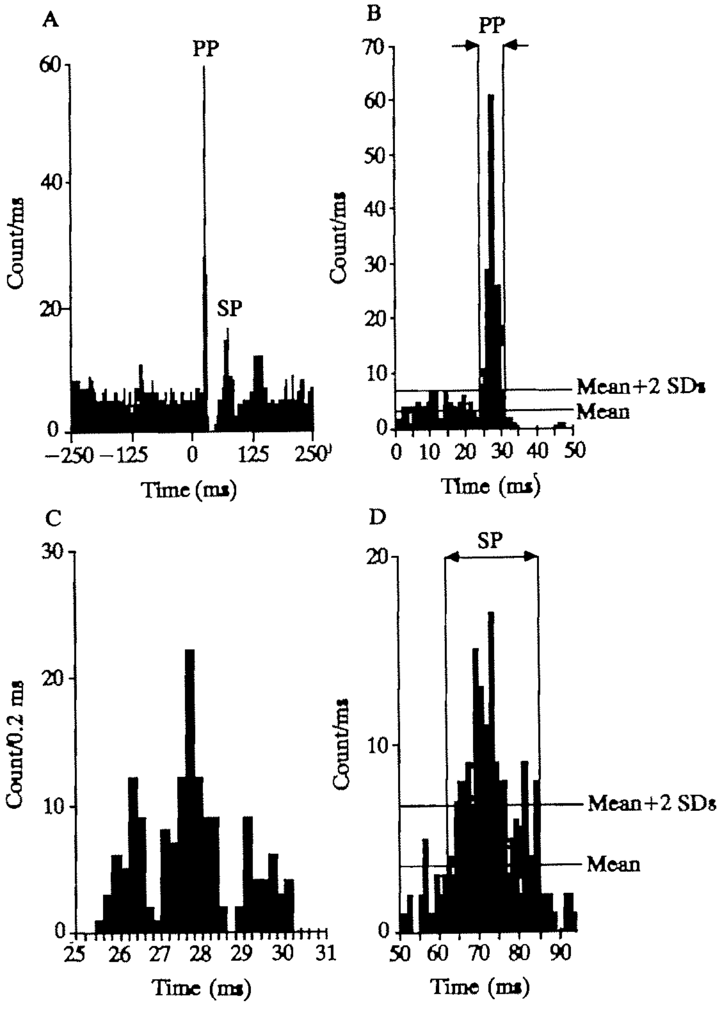

FIG. 2 A, PSTH constructed with a bin width of 1 ms of the discharge of a single motor unit from a healthy subject in response to 500 magnetic stimuli at the vertex. The stimulus is at time zero, as in all the illustrated PSTHs. The primary (PP) and secondary (SP) peaks are indicated. B, PP was considered to be present if filled bins intersected a line drawn at 2 SDs above the mean firing level calculated from the 250 ms before the stimulus. The latency and duration of PP were calculated from the intersection of the peak with the mean prestimulus firing level. C, PSTH of the same motor unit constructed with a bin width of 0.2 ms and showing 3 subpeaks. All subpeaks had a peak count of at least 10 times the mean prestimulus firing level. Intermodal intervals were calculated from the midpoint times of the peak bin counts within each subpeak. D, SP was considered to be present if filled bins intersected a line drawn at 2 SDs above the mean prestimulus firing level. The onset and offset of SP were then defined by the points at which the bin count fell below the mean prestimulus firing level for more than 2 consecutive bins.

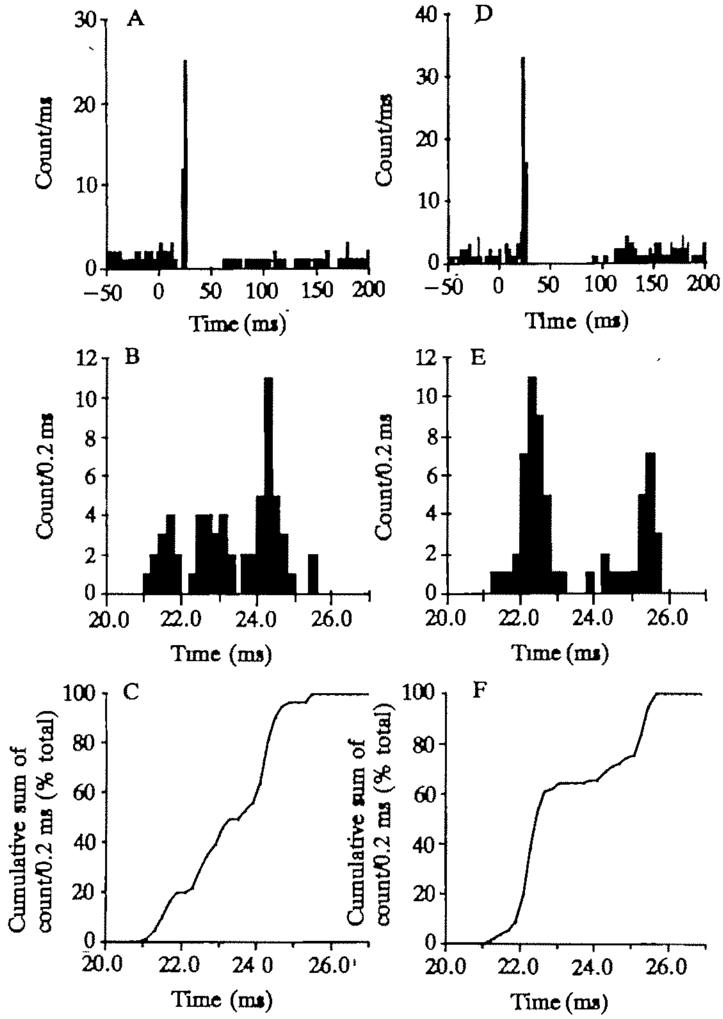

FIG. 3. Analysis of the discharge within PP in a healthy subject (A, B, C, 93 trials) and in a patient with MS (D, E, F, 120 trials). PSTHs constructed with a bin width of 1 ms (A, D) and 0.2 ms (B, E) are shown, with the corresponding cumulative sums normalized to the total number of discharges within PP (C, F). Three distinct subpeaks are seen in B (with intermodal intervals of 1.0 and 1.6 ms) and 2 subpeaks in E (with an intermodal interval of 3.2 ms). The contrasting shapes of the cumultive sums are evident in C and F.

1.4 (±0.4) ms (n = 26) and ranged from 0.6 to 2.4 ms. The mean (±SD) intermodal interval between first and second subpeaks was 1.3 (±0.4) ms (n = 17) and between second and third subpeaks was 1.4 (±0.4) ms (n = 9). In 5 motor units only a single narrow peak was present with PP. In 4 motor units, subpeaks within PP could not be discerned with certainty. The rank order of subpeaks within PP had no obvious influence on their peak height.

Stimulus intensity. With FDI relaxed, the stimulus intensity at which a just discernible compound muscle action potential recorded with surface electrodes over FDI could be obtained ranged from 36 to 54%. With unitary recording, only a limited range of stimulus intensities could be investigated because higher intensities resulted in compound motor unit potentials within which the index motor unit spike could not be discriminated. Increasing intensities were studied in 7 motor units, using both the random and spike triggered protocols. The main effect observed was that the proportion of trials in which the motor unit fired within PP increased, but a maximum could not be found with the range of intensities used. In 1 motor unit, in which stimuli were given at a fixed interval of 60 ms after the previous voluntary spike, a higher intensity produced additional subpeaks later in the PSTH (fig. 4). In another MU, random stimuli at a lower stimulus intensity produced 2 subpeaks with peak latencies of 30.7 and 31.5 ms, but when a higher stimulus intensity was used, only a single peak with a peak latency of 29.8 ms was present.

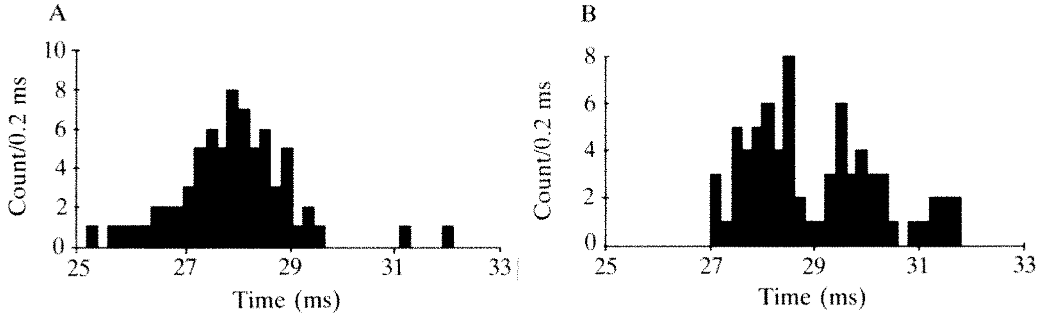

FIG. 4. Results from a spike-triggered stimulation experiment in which stimuli at 2 intensities were given 60 ms after a triggering motor unit spike in a healthy subject. A, a stimulus intensity of 32% has produced a single peak with a modal latency of 28.0 ms (188 trials). B, a stimulus intensity of 42% has produced 2 additional subpeaks at longer latencies, with intermodal intervals of 1.0 and 2.0 ms (99 trials).

The influence of the previous voluntary discharge. That the timing of the preceding voluntary discharge influenced whether or not the motor unit fired within PP is seen in fig. 5A. In 6 motor units the intervals between the preceding voluntary discharge and the stimulus (spike-to-stimulus intervals) were grouped into 10 ms bins to relate the percentage of trials in which a PP spike occurred to the spike to stimulus interval. The excitability of the motoneuron expressed as the probability of it producing a spike in PP was seen to increase as time elapsed from the previous voluntary spike (fig. 6A). In the 6 motor units, stimuli at the intensities used, falling within 0–30 ms of the previous spike were unlikely to bring the motoneuron to threshold, whereas those falling later produced greater probabilities of firing. In all 6 motor units, the proportion of PP trials exceeded 20% at spike-to-stimulus intervals greater than 40 ms.

In random stimulation experiments, the latency of motor unit discharge within PP was in 7 of 10 motor units significantly correlated with the spike-to-stimulus interval (fig. 7A), such that stimuli falling later after the previous spike tended to be associated with earlier discharge within PP. Using the spike triggered stimulation technique, the

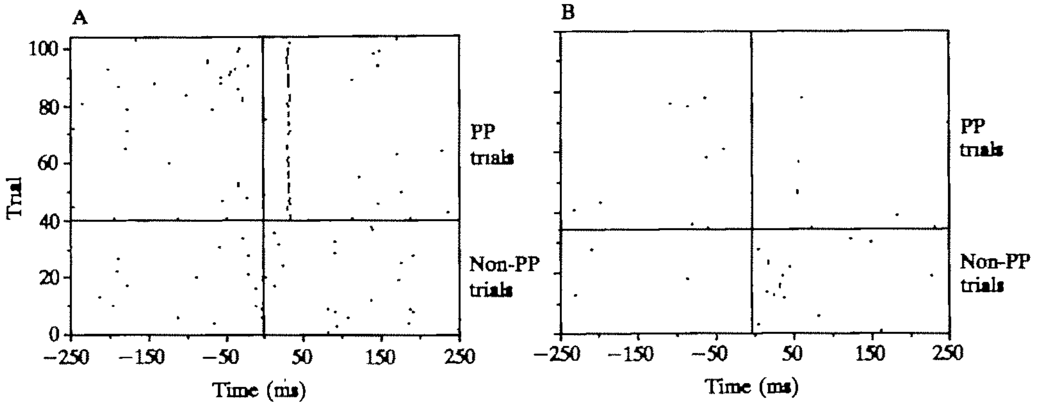

FIG. 5. Sorted raster plot of the discharges of a single motor unit from a healthy subject (A, 103 trials) and *Case 1* (B, 99 trials, motor unit A2). Each point represents a single discharge. Magnetic stimuli were given at time zero. Trials have been separated into those with a discharge within PP (*upper panel*) and those without (*lower panel*). Differences in the firing times of spikes before the PP latency in the 2 panels are clearly seen in A and B, with a broader cluster of PP discharges occurring at a longer latency seen in B.

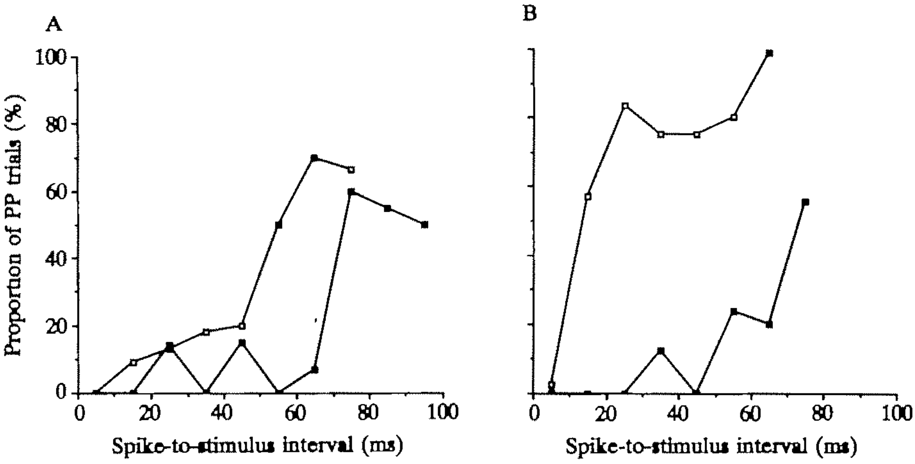

FIG. 6 In random stimulation experiments, the intervals between the stimulus and the preceding discharge (spike-to-stimulus intervals) have been collected into 10 ms bins to relate this interval to the proportion of trials in which a PP spike occurred. Each curve represents the minimum and the maximum rate of rise observed for each of the 2 subject groups. A, 2 motor units from a healthy subject (filled squares = 168 trials, mean ISI 146.4 ms; open squares = 155 trials, mean ISI 124.9 ms). B, 2 motor units from patients with MS (2-1 = filled squares: 170 trials, mean ISI 143.6 ms; 3-2 = open squares: 70 trials, mean ISI 103.8 ms). Spike-to-stimulus intervals greater than the minimum ISI for each motor unit are not shown. All motor units show a rise in excitability with time, with a greater variability between different ones observed in MS patients.

longer the delay from the triggering spike to the stimulus, then the greater was the probability of producing a spike in PP. If the delay was shorter, the motor unit fired on a smaller proportion of trials but now did so at a longer latency (fig. 8).

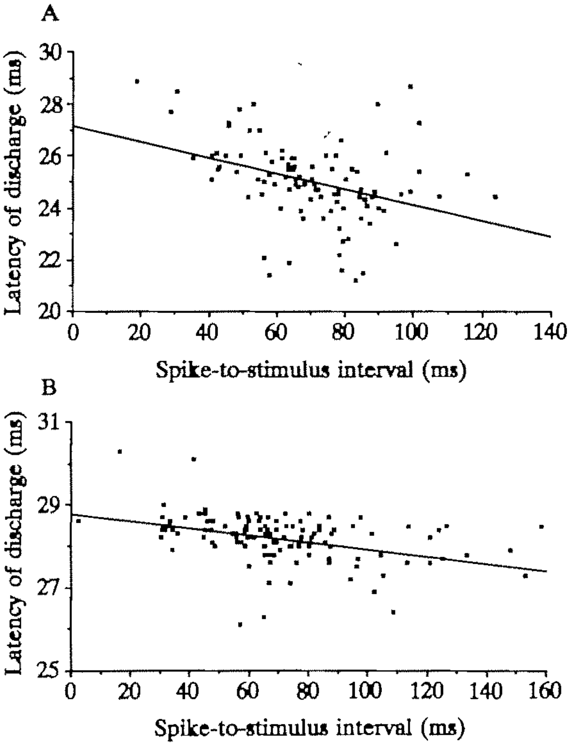

Fig 7. The latency of discharge of a single motor unit within PP is significantly correlated with the interval between the stimulus and the preceding discharge (spike-to-stimulus interval). The longer the interval then the earlier is the discharge within PP A, healthy subject (r = 0.139, $P < 0.001$, 492 trials, 96 PP trials). B, motor unit 6-2 from an MS patient (r = 0.116, $P < 0.001$, 276 trials, 118 PP trials).

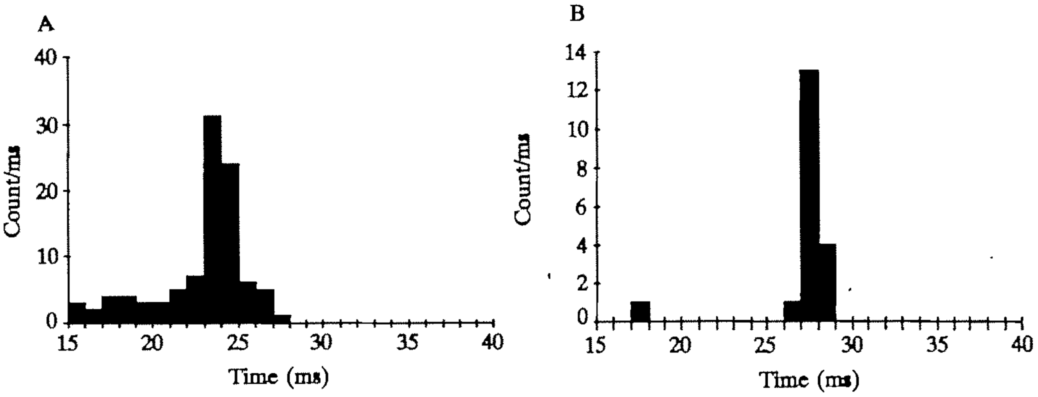

Fig. 8. PSTHs from a spike-triggered experiment on a motor unit in a healthy subject with stimuli given at 2 delays after the previous voluntary spike. A, stimuli were given at 80 ms after the previous voluntary spike and have produced a PP at a peak latency of 23.5 ms (125 trials). B, identical stimuli were given at a delay of 10 ms after the previous voluntary spike and have produced a PP at a latency of 27.5 ms (274 trials).

Patients with MS

In 19 of the 21 motor units in MS patients, the firing probability changed after the stimulus when compared with the mean prestimulus firing level. The features of these changes are summarized in Table 2.

TABLE 2 CHARACTERISTICS OF MOTOR UNITS IN PATIENTS

Case-motor unit	*Side studied*	*Stimulus intensity (% max.)*	*Surface threshold (% max)*	*Primary peak*		*No. of subpeaks*	*Intersubpeak interval (ms)*
				Onset (ms)	*Duration (ms)*		
1-1	R	59	65	59	21	2	7
1-2	R	59	65	51	18	2	22
2-1	R	55	51	23	7	2	2.6
2-2	R	60	51	21	5	2	3 2
2-3	R	60	51	23	1	1	–
3-1	L	73	>80	Absent		–	–
3-2	R	50	45	33	16	2	8
4-1	R	67	70	35	20	3	8 5 and 7.5
5-1	L	72	90	69*	39	–	–
5-2	L	72	90	83*	15	–	–
5-3	R	72	90	Absent		–	–
6-1	L	52	67	27	2	1	–
6-2	L	52	67	26	4	1	–
7-1	L	70	NA	71*	27	–	–
7-2	L	70	NA	29	4	1	–
8-1	R	43	54	34	9	2	6
8-2	R	43	54	30	13	2	8
8-3	R	43	54	40	5	1	–
9-1	R	70	99	38	9	3	2.4 and 2.4
9-2	R	70	99	37	8	2	8
9-3	R	68	99	34	13	–	–

* Possibly secondary peak (*see text*). NA = not available.

Stimulus intensity. With FDI relaxed, the stimulus intensity at which a just discernible compound muscle action potential recorded by surface electrodes could be obtained was frequently higher for patients with MS, ranging from 45 to 99%, when compared with a range of 36–54% in healthy subjects. Case 3, with a threshold of 45% for the right FDI, had a threshold of >80% for the left FDI which was the clinically more severely affected side. Similarly, on studying single motor units in this patient, a stimulus intensity of 50% produced a PSTH with a PP from the right side, whereas an intensity of 73% produced no excitatory response on the left. In Case 5, the threshold intensity for surface responses was 90% on both sides, which were equally affected clinically. A stimulus intensity of 72% produced no excitatory response in the PSTH for a motor unit in the right FDI (*see* fig. 10A), but the same intensity produced PSTHs from 2 motor units with late peaks in the left FDI.

The primary peak. The observations made here on motor units in patients with MS principally concern PP, rather than SP. With PSTHs constructed with a bin width of 0.2 ms, the intermodal interval between subpeaks in 3 motor units was 2.6 ms (2-1), 3.2 ms (2-2), and 2.4 and 2.4 ms (9-1) (*see* fig. 3E). In the motor units from patients exhibiting such subpeaks the intermodal interval was therefore equal to, or greater than, the maximum interval observed in a healthy subject (2.4 ms). The occurrence of other multiple peaks with prolonged intervals are described below.

In 9 motor units (1-1, 1-2, 3-2, 4-1, 8-1, 8-3, 9-1, 2, 3) the onset latency of those peaks which had the characteristic shape of PP was delayed by 2 to 28 ms with respect to the longest onset latency observed in healthy subjects (31 ms). In 5 of these 9 motor units, the duration of PP was increased by 4 to 12 ms, when compared with the PP of longest duration observed in a healthy subject (9 ms). In 6 of the same 9 motor units, plus 1 other (8-2), the PP region of the PSTH, when constructed with a bin width of 1 ms, was found to consist of a principal component which was either preceded or followed by an additional one or two peaks of smaller height (fig. 9A, C, E). The height of each of these peaks exceeded the mean prestimulus firing level +2 SDs and the firing probability fell to zero between peaks. Intervals of 6 – 8.5 ms separated these successive peaks, with one interval of 22 ms (1-2). This was not caused by multiple firings of the motor unit within the same trial, and was never found in healthy controls. These peaks had no discernible subpeak structure when examined with 0.2 ms bins (*see* fig. 9B, D, F). In 3 motor units (7-1, 5-1, 5-2) a single peak of increased firing probability was found at latencies of between 69 and 83 ms and with durations of between 15 and 39 ms.

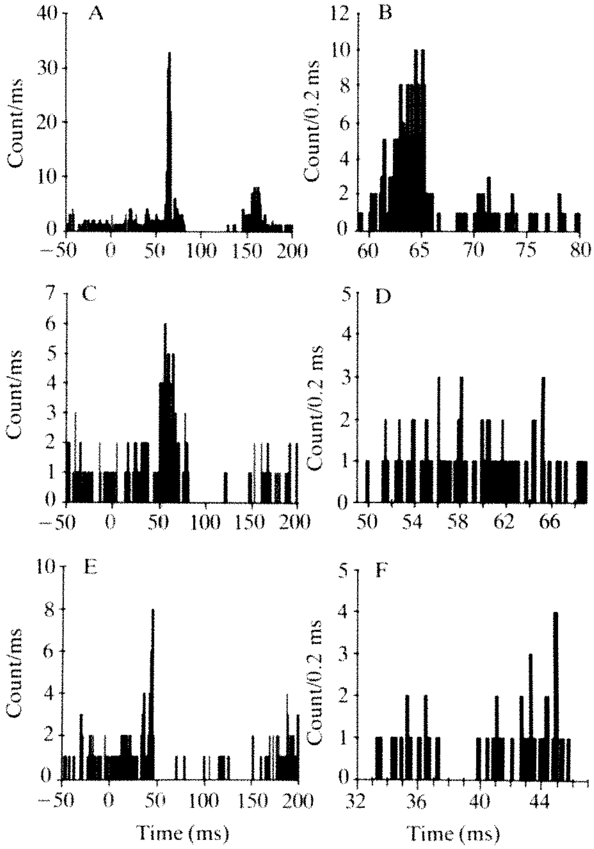

FIG. 9. PSTHs from 3 motor units (1-1, 1-2, 3-2) in 2 MS patients constructed with a bin width of 1 ms (A, C, E) and 0.2 ms (B, D, F) showing the features of PP. A, PP has an onset latency of 59 ms and a duration of 21 ms containing a principal peak followed by 2 much smaller peaks, each separated by periods of zero firing probability (as seen in C) and each exceeding the mean prestimulus firing level plus 2 SDs (motor unit 1-1, 221 trials). B, PP region from A, showing lack of distinct subpeaks within the principal component of PP. C, PP has an onset latency of 51 ms and a duration of 18 ms, the principal component of which is followed by a peak of smaller height and duration (motor unit 1-2, 99 trials). D, PP region from C, showing lack of distinct subpeaks. E, PP has an onset latency of 33 ms and a duration of 16 ms, comprising 2 peaks, each of which lacks distinct subpeaks, as seen in F (motor unit 3-2, 70 trials).

PPs of normal latency and duration, with single subpeaks comparable with those found in healthy subjects, were found in 3 motor units (6-1, 6-2, 7-2) (fig. 10B, C). By contrast, another motor unit (7-1) from the same muscle in 1 of these patients had a PSTH

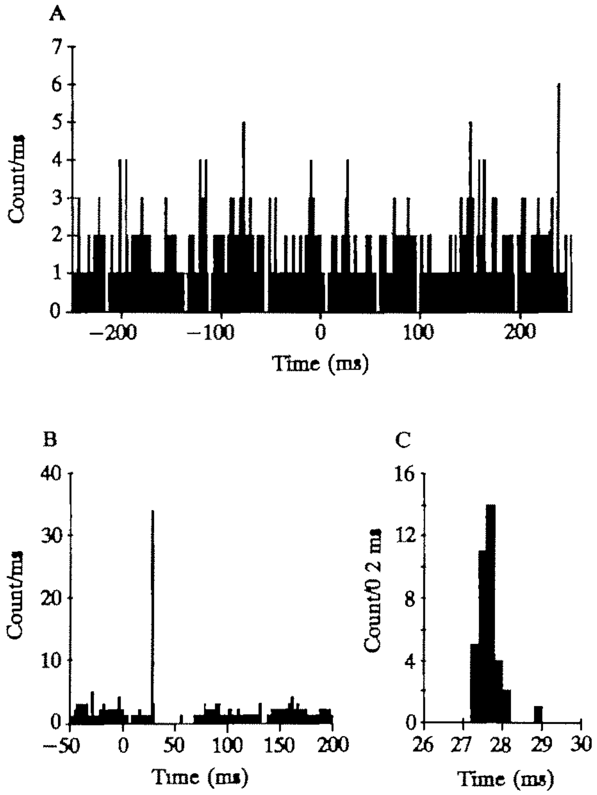

FIG. 10. PSTHs from 2 MS patients constructed with a bin width of 1 ms (A, B) and 0 2 ms (C) contrasting the features of an absent excitatory response with a normal response. A, PSTH plotted from −250 to +250 ms with respect to the stimulus, which was given at time zero. No rise in firing probability (confirmed with a cusum with limits of ±2 SD) is seen following the stimulus (motor unit 3-3, 120 trials, stimulus intensity 72%). B, PP has an onset latency of 27 ms and a duration of 2 ms, comprising a single distinct subpeak, as seen in C (motor unit F1, 95 trials, stimulus intensity 52%).

containing a single peak of 27 ms duration with an onset latency of 71 ms. Of the 9 patients studied, this was the only example where motor units exhibiting both normal and abnormal features were found in the same muscle.

The influence of the previous voluntary discharge. The influence of the spike-to-stimulus interval on the probability of producing a PP discharge (fig. 5B) was studied in 9 motor units in the same way as for those from healthy subjects. The excitability displayed a similar change with time (fig. 6B). It was initially relatively low and then showed a rise at longer spike-to-stimulus intervals. The variability in the time course of this change between different motor units was, however, greater than that found in healthy subjects. Also in contrast to controls, the proportion of PP trials in 8 of 9 motor units had already exceeded 20% at spike-to-stimulus intervals of 40 ms.

The latency of the motor unit discharge within PP was in 7 of 10 motor units significantly correlated with the interval between the stimulus and the preceding voluntary discharge (fig. 7B).

DISCUSSION

All motor units studied were of low threshold and discussion must be limited to this subpopulation of the motoneuron pools. In healthy subjects all units showed a brief short-latency rise in firing probability in response to magnetic stimulation. It will be argued that this represents operation of a monosynaptic corticospinal pathway and that a sequence of EPSPs is initiated at the motoneuron. Factors influencing these events in healthy subjects and the changes observed in patients with MS will be discussed.

The relationship between the form of PSTHs and the underlying membrane events has been investigated directly in the cat (Fetz and Gustafsson, 1983; Gustafsson and McCrea, 1984) and by computer models (Knox, 1974; Kirkwood and Sears, 1978; Ashby and Zilm, 1982*a*). It appears that peaks in PSTHs are related both to the size of the underlying EPSP and to its first differential, but provided that EPSPs are large (>3 mV) then the peak in a PSTH corresponds most closely to the first differential of the rising phase of the EPSP. It follows that the duration of a peak would approximate to the rise time of the EPSP.

In the present experiments, the shape of peaks in the PSTH is also affected by the membrane trajectory of the motoneuron between discharges. A motoneuron voluntarily firing with a mean ISI of say 110 ms may have ISIs ranging from 80 to 140 ms. Assuming that the membrane potential rises linearly following the after-hyperpolarization and that the super-added EPSP has a maximal rise time of 7.5 ms (Kernell and Wu, 1967*b*), it can be calculated that the contribution of ISI variation to the dispersion of discharges within PP would be up to about 3.2 ms. Thus we believe that the dispersion and filling in of histogram bins between subpeaks could be attributable to ISI variation in addition to variations in EPSP amplitude and rise time. The same cannot be said, however, of subpeak maxima which would continue to reflect the point of maximum velocity of EPSP rise, irrespective of variations in ISI. The intermodal intervals of subpeaks found in healthy subjects in the current experiments correspond closely to values found in primates for the intervals between successive corticospinal impulses induced by cortical stimuli (Kernell and Wu, 1967*a*). The mean interval of 1.4 ms between neighbouring subpeaks implies that single cortical shocks can cause corticospinal fibres to discharge at rates in the region of 700/s.

Since subpeaks are dispersed for reasons other than the rising phase of the underlying EPSPs, the rise times of EPSPs inferred from PSTHs will be overestimated. Indeed, the cumulative sums constructed from PSTHs represent the combined effects of EPSP shape and ISI variations. The rise time of one component of the underlying EPSP, however, must be less than the intermodal interval between subpeaks. The values of 0.6–2.4 ms compare well with the rise time quoted for operation of a monosynaptic connection (Kirkwood and Sears, 1982).

It was a clear finding that the interval between the previous voluntry spike and the stimulus had a major influence on whether or not the motor unit fired in PP. This interval also influenced the time of firing within PP, with longer spike-to-stimulus intervals being associated with earlier firings. This may indicate that the motoneuron membrane potential at a given time interval after a discharge also determines which single EPSP of the sequence will bring the cell to firing threshold; at later stages of the ISI earlier EPSPs will cause the cell to fire. Ashby and Zilm (1982*b*) investigating the Ia input to human lumbar motoneurons found that the time of arrival of the afferent volley determined whether or not the motor unit fired, implying a gradual rise in excitability between voluntary discharges. In the cat, it has been shown that steady current injection into motoneurons (Schwindt and Calvin, 1972), such as might be envisaged if human motoneurons are subject to a descending drive, causes repetitive motoneuron firing and that the membrane potential follows a scoop and ramp sequence between discharges. Despite abnormalities in their PSTH, some motoneurons in MS patients displayed behaviour that was similar to those in healthy subjects in terms of the change in their excitability with time after a voluntary discharge. This would suggest that the normal intrinsic processes that govern excitability operate in these motoneurons. The observation that in 8 of 9 motor units PP discharges could be induced at an earlier stage of the ISI than in healthy subjects may reflect an alteration in the time course of the motoneuron membrane trajectory, or the production of larger EPSPs, resulting either from the required use of higher stimulus intensities for the production of PPs in MS patients, hyperexcitability of presynaptic elements or from changes in the postsynaptic membrane.

The most common difference between patients and healthy controls was an elevation in the threshold intensity, both at which surface recorded responses and at which unitary responses could be detected. In 1 patient the difference in threshold between the two sides corresponded to the severity of the upper motor neuron signs. Furthermore, in 2 motor units, it was found that despite using a stimulus intensity well above that which produced a PP in healthy subjects, no excitatory response in the PSTH could be detected. One possibility for these findings is that the cerebral neurons usually activated by such stimuli have high thresholds for activation because of demyelination of fibres; it is well known that peripheral nerve fibres which have been demyelinated also have high thresholds. Alternatively, the number of effective corticospinal inputs to the motoneuron could be depleted (fig. 11D), or the overall excitability of the motoneuron reduced.

Raised thresholds for activating single motor units might also be expected to occur with upper motor neuron lesions caused by diseases other than MS, including motor neuron disease and stroke. Indeed, this may be inferred from studies of surface recorded compound muscle action potentials (CMAPs) following transcranial stimulation in these conditions (Berardelli *et al.*, 1987; Ingram and Swash, 1987). Using electrical transcranial stimulation, subcortical infarcts have been found to be associated with CMAPs of delayed onset (MacDonell *et al.*, 1989). Delayed CMAPs following magnetic transcranial stimulation have also been found in motor neuron disease (Ingram and Swash, 1987; Schriefer *et al.*, 1989) and in MS (Hess *et al.*, 1987*c*).

In some motor units in patients with MS, PP was delayed by up to 28 ms when compared with healthy subjects. In these motor units, PP was also of longer duration than found in healthy subjects. The fact that the response is delayed could be due to demyelination and therefore slowed velocity of propagation in corticospinal fibres. Slowing, per se, would not be expected to cause dispersion of PP, only its late arrival, but this assumes that the propagation velocities in the many corticospinal fibres which project to a single motoneuron are slowed to the same extent. It is more likely that such corticospinal fibres are variably demyelinated, with propagation velocities that are variably reduced (*see* fig. 11B). A combination of these factors may therefore have caused the late arrival and the increased duration of excitatory effects observed in these motoneurons. The absence of discernible subpeaks in some motor units would also indicate the

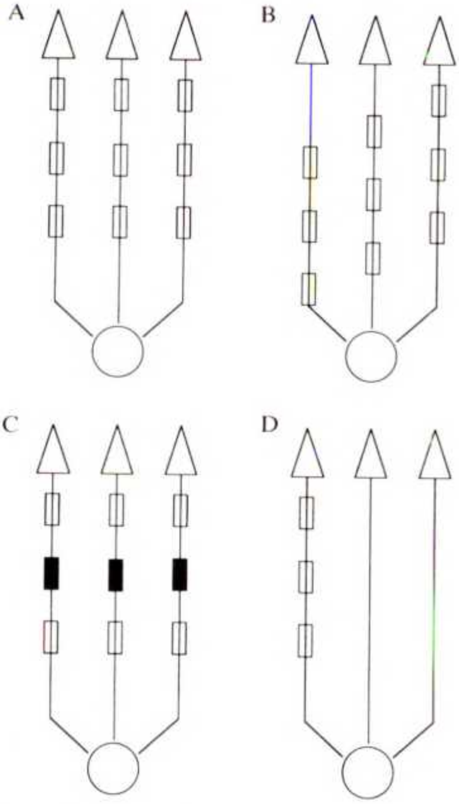

FIG. 11. Diagram to illustrate the mechanisms postulated to underlie the response of single spinal motoneurons to a single transcranial magnetic stimulus in healthy subjects (A) and in MS patients (B, C, D). A, the descending fibres from corticospinal neurons (triangles) converge on to a spinal motoneuron (circle). Three trains of impulses (open blocks) in each fibre descend as 3 synchronized volleys. B, variable degrees of slowing in the velocity of propagation desynchronize the trains of impulses within each volley. C, the intermediate volley (filled blocks) is either absent or fails to conduct (frequency-dependent conduction block). D, normal conduction in 1 fibre, with absence of descending impulses in the 2 other fibres which may be due to either a failure of the corticospinal neuron to respond to the stimulus, or absolute conduction failure.

desynchronization of descending impulses by varying degrees of demyelination between different corticospinal fibres. Desynchronization would lead to less effective temporal summation of EPSPs at the motoneuron which, in some cases, might never reach firing threshold.

In a series of patients with upper motor neuron lesions including MS, motor neuron disease and stroke, studied with electrical transcranial stimulation, the greatest delay in the onset of the CMAP was found in patients with MS (Thompson *et al.*, 1987). This delay was also associated with an increased duration of the response in MS, but not in motor neuron disease. The authors' suggestion that a delay in the onset of CMAPs may be caused by slowing of conduction in the largest diameter fibres of the pyramidal tract, or dispersion or reduction in size of the descending volley is supported by the present findings in single motor units.

In some motor units it was found that, although PP was essentially of normal latency and duration, the subpeak structure was disturbed such that the interval between adjacent subpeaks was prolonged by up to 0.8 ms when compared with the longest intersubpeak interval found in a healthy subject. Motor units with multiple peaks at more prolonged interpeak intervals than this were associated with PPs of delayed onset and increased duration (*see below*). It has been argued that subpeaks represent the EPSPs caused by the successive arrival of corticospinal impulses at the motoneuron. The prolongation of intervals between neighbouring subpeaks in MS patients suggests that a single subpeak may be missing. It is possible to attribute this to the inability of partially demyelinated central fibres to conduct all the corticospinal impulses in the high-frequency train produced by the stimulus. Demyelinated fibres, after conducting the first impulse in a train, might fail to conduct the second, but would have recovered sufficiently to conduct the third. Such frequency-dependent conduction block has been demonstrated in the cat posterior column where, with frequencies of from 290 to 410/s, alternate impulses failed to cross a demyelinated lesion (McDonald and Sears, 1970). It would not be surprising then for corticospinal fibres which may conduct trains up to 700/s to be subject to the same phenomenon (fig. 11c). Alternatively, missing subpeaks could be attributed to a differential threshold for successive I waves (Kernell and Wu, 1967*a*; Day *et al.*, 1989) although evidence for this was not observed in the current experiments on healthy subjects.

The presence of more than one peak in the PP region of PSTHs from 7 of the 21 motor units, at intervals of 6–8.5 ms (or 22 ms in 1 motor unit), frequently associated with PPs of delayed onset, prolonged duration and absence of subpeaks with normal features could indicate an even greater degree of frequency-dependent conduction block. The duration of the block would be equivalent to at least the interval between the peaks, following which a late volley of sufficient magnitude, possibly caused by the use of a high stimulus strength, would fire the motor unit. These peaks may also, however, represent the operation of more than one physiological pathway or the same pathway that had become partially demyelinated producing a discrete division into fibre groups with different conduction velocities. The sprouting of corticospinal neurons on to partially denervated motoneurons could produce new pathways involving a different number of synapses. Intraspinal sprouting of dorsal root axons may follow partial denervation in the cat (Liu and Chambers, 1958), and morphological reorganization of the spinal cord rostral to the site of hemisection in the monkey and rat has been demonstrated with similar changes in a patient with a spinal cord lesion (Bernstein and Bernstein, 1973). Pathological loss of effective excitatory synapses, and/or axonal sprouting to form new synapses may have contributed to changes in the time course of the EPSP underlying PP. Alternatively, the period of reduced firing probability occurring between the peaks could represent an inhibitory process acting during the course of a temporally dispersed PP, or such multiple peaks may have been produced by a change in the mechanism that is thought to produce repetitive corticospinal cell firing in response to a single stimulus (Jankowska *et al.*, 1976; Phillips, 1987).

ACKNOWLEDGEMENTS

Financial support from the Medical Research Council and the Multiple Sclerosis Society of America is gratefully acknowledged. S.J.B. is an MRC Training Fellow. M.S. is supported by the Deutsche Forschungsgemeinschaft.

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(*Received November 17, 1989. Revised April 4, 1990. Accepted April 20, 1990*)

Brain (1991), **114**, 663–681

CORRELATION OF SEP ABNORMALITIES WITH BRAIN AND CERVICAL CORD MRI IN MULTIPLE SCLEROSIS

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SUMMARY

In 31 patients with definite or suspected multiple sclerosis (MS) presenting with a cervical cord syndrome, somatosensory evoked potentials (SEPs) were recorded to median and posterior tibial nerve stimulation, using cephalic and noncephalic reference electrodes. Magnetic resonance imaging (MRI) of the brain and cervical spinal cord was performed, the latter in sagittal and axial views. SEPs were abnormal in 67.7% of patients, whereas MRI showed cervical cord lesions in 74.2% and intracranial lesions possibly involving the somatosensory pathways in 64.5% of cases. A significant correlation was found between abnormalities of cervical (N13) and cortical (N20) potentials following median nerve stimulation with Fz reference and MRI abnormalities involving the ipsilateral or posterior half of the cervical cord, but not the contralateral or anterior half. The N13 potential, recorded from the low cervical region to a supraglottal reference, was most frequently abnormal in patients with MRI lesions at C6 or C7, whereas P14, recorded from the scalp to a clavicle reference, was most often affected by lesions at C1 or the cervicomedullary junction. Abnormalities of the cortical P40 to tibial nerve stimulation were less significantly correlated with cervical MRI lesions. The latency of N20 measured from N9 at the clavicle and the absolute latency of P40 were significantly correlated with the length of MRI abnormalities in the ipsilateral cervical cord. No significant correlation was observed between SEP abnormalities and brain MRI lesions, which it was considered might possibly involve the intracranial somatosensory pathways. It was concluded that (1) the morphological lesions seen in MRI of the cervical cord usually give rise to appropriate electrophysiological deficits, but the occasional finding of a widespread MRI lesion with normal SEP suggests that myelin damage is not the only or the major factor responsible for abnormal MRI signal; and (2) 'clinically silent' lesions apparently involving the radiations and other sensory structures of the brain appear not to give rise to detectable SEP abnormalities, using the methods of the present study

INTRODUCTION

The physiological properties of nerve fibres are to a large degree dependent on the integrity of their myelin sheaths. Experimental demyelination causes either slowing or blockage of conduction (McDonald, 1963). When an electrical stimulus is applied to a nerve trunk and recording electrodes are placed at appropriate locations over the neck and scalp, somatosensory evoked potentials (SEPs) can be recorded, which reflect the passage of the afferent volley at different levels of the somatosensory system. In the presence of a demyelinating process affecting sensory nerve fibres, defects in axonal conduction can frequently be detected as delay, reduction or absence of the SEPs (*see* Jones, 1982*a* for review).

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Previous studies have demonstrated a high incidence of abnormal SEPs in patients with suspected or clinically definite multiple sclerosis (MS), with or without deficits of proprioceptive or discriminative tactile sensation (Baker *et al.*, 1968; Small *et al.*, 1978; Gambi *et al.*, 1982). The demyelinating lesions of MS can be detected by magnetic resonance imaging (MRI) in the brain (Young *et al.*, 1981) and spinal cord (Maravilla *et al.*, 1984). These changes usually appear as focal areas of altered signal and are most often located in the deep cerebral white matter. Previous investigations have demonstrated MRI abnormalities in a high percentage of patients with MS, in both the early and advanced stages of the disease (Young *et al.*, 1981; Lukes *et al.*, 1983; Runge *et al.*, 1984; Ormerod *et al.*, 1987; Honig *et al.*, 1988).

Whereas the diagnostic usefulness of SEPs and MRI in MS has been widely investigated and is now well established, little is known about the correlation between the abnormalities shown by these techniques. In this study we examined 31 patients with a cervical cord syndrome, believed to be due to a demyelinating lesion. In some cases this was clinically isolated and in others it appeared associated with clinically disseminated deficits; in the latter the criteria for MS, established by Poser *et al.* (1983), were fulfilled. SEPs were recorded from the upper and lower limbs and compared in detail with MRI of the cervical spinal cord and brain. SEPs and MRI findings were also compared with the pattern of clinical abnormalities.

MATERIAL AND METHODS

Patients

The patient group consisted of 31 subjects, 21 females and 10 males, aged 22–51 (mean 35.4) yrs. Each patient presented with a cervical cord syndrome, involving the sensory and/or motor pathways, and 12 also showed clinical evidence of lesions located elsewhere in the central nervous system (CNS). When clinical features, evoked responses, cerebrospinal fluid analysis for oligoclonal IgG bands and MRI findings were all considered, 27 patients were classified by the Poser criteria (Poser *et al.*, 1983) as having MS: clinically definite MS, 3 patients; laboratory supported definite MS, 11 patients; clinically probable MS, 13 patients. The other 4 cases, who did not show evidence of abnormalities except in the cervical spinal cord, were classified as having an isolated cord lesion. the clinical and radiological findings in these patients provided no evidence of traumatic, compressive, degenerative or vascular pathology and the lesion was therefore considered compatible with demyelination. The cord syndromes had at the time of study a duration of 1–139 (mean 31.7) months and appeared relapsing and remitting in 21 cases, progressive (duration longer than 6 months without remission or exacerbation) in 7 cases and acute (1 episode lasting less than 1 month) in 3 cases.

Controls

The SEP control group consisted of 31 healthy volunteers, 16 males and 15 females, aged 18–43 (mean 29.9) yrs.

SEPs

Two or more SEP recordings were obtained from each stimulated limb in each subject. The stimulus (200 μs constant voltage square wave) was delivered at 3.2 impulses/s to the median nerve at either wrist and the tibial nerve at either ankle, with an intensity sufficient to produce a moderate twitch of the thenar and plantar muscles respectively.

Responses were recorded by silver/silver chloride disc electrodes, located over the midpoint of each clavicle, the spinal vertebrae C7 and C2, the anterior aspect of the neck on the midline above the thyroid cartilage ('supraglottal' location), the mastoid processes, both hand areas of the sensory cortex (2 cm posterior to the vertex and 7 cm towards each tragus) and the foot area of the sensory cortex (2 cm posterior to the vertex); all the active electrodes were referred to Fz (International 10-20 System). Later in off-line analysis, the supraglottal waveform was subtracted from the C7 response and the clavicle response (contralateral to the stimulated arm) was subtracted from all channels, in order to obtain noncephalic-referred neck and scalp waveforms.

The amplifiers' high frequency response was less than 3 dB down at 5 kHz and the time constant was 1 s Four hundred sweeps were averaged in each run. In recordings from the median nerve the averager epoch was 32 ms (8 sample points/ms) starting 3 ms after the stimulus pulse, and in recordings from the tibial nerve the epoch was 64 ms (4 sample points/ms) with a 13 ms poststimulus delay. When it was impossible to identify a significant cortical response within this period, the averager epoch was doubled (64 or 128 ms, respectively).

Using the Fz reference, the major components evoked from the median nerve were N9 (clavicle), N13 (C7) and N20 (scalp), whereas from the tibial nerve the main component recorded was P40 (scalp) The responses obtained in the noncephalic reference waveforms were N13 (C7-supraglottal) and P14 (scalp-clavicle) (fig. 1). N9-N20 and N13-N20 interpeak latencies were measured in the Fz reference recordings, but only the former was used for statistical evaluation, as N13 in some waveforms was abnormally degraded and its latency difficult to measure.

The normal latency limit for each SEP component was defined by the mean+2.5 SD of the absolute latency, interpeak latency and left-right latency difference in the control group. The amplitude limit was defined by the mean−2.5 SD of log amplitude, log (left/right) and log (P14/P9) amplitude ratio (the latter as suggested by Garcia Larrea and Mauguière, 1988).

MRI

All patients were imaged on a Picker 0.5 Tesla superconducting scanner within 10 days of the SEP recordings. Brain MRI was interpreted by one author (G.H. du B.) and cord MRI by another (D.H.M.), both of whom were blind to the electrophysiological data. Lesions were defined as areas of unequivocally increased signal on T_2-weighted sequences or of decreased signal on T_1-weighted sequences.

The brain was imaged using a 30 cm spherical receiver coil. A moderately T_2-weighted sequence ($SE_{2000/60}$) was performed in every case and, when changes were equivocal, a more heavily T_2-weighted sequence ($SE_{2000/120}$) and/or a T_1-weighted sequence ($IR_{2000/40/500}$) was added. Contiguous 5 or 10 mm thick axial slices were obtained through the whole brain.

A saddle-shaped surface receiver coil was provided by the manufacturer to image the cervical spinal cord. Contiguous 5 mm thick sagittal slices through the cord were obtained in every case using T_1 ($SE_{500/40}$) and T_2-weighted ($SE_{1500/80}$) sequences. Additional contiguous axial 5 mm slices were obtained through regions where intrinsic abnormalities (i.e., high signal foci on the $SE_{1500/80}$ sequence) were suspected on the sagittal image. Abnormalities were accepted only when identified on both sagittal and axial images. The longitudinal extent of the lesion between the cervicomedullary junction and C7 was determined from sagittal images. The transverse extent of the lesion was classified from axial images, by dividing the cord into 4 quadrants: left anterior and posterior, and right anterior and posterior. A lesion was said to be present in a given quadrant when an area of altered signal occupied the greater part of the quadrant. The resolution of the imager did not allow identification of individual fibre tracts within the cord. In correlating the morphological, clinical and electrophysiological data, an assumption has been made that lesions in a posterior quadrant of the cord probably involve the posterior columns, while lesions in an anterior quadrant are likely to involve the spinothalamic tracts.

RESULTS

Clinical findings

The patients with cervical cord syndromes included 24 with involvement of both sensory and motor systems, 4 with isolated sensory and 3 with isolated motor impairment. The sensory symptoms consisted of numbness and paraesthesiae; the sensory signs suggested lesions in the posterior columns (abnormality of joint position sense, two-point discrimination or vibration sense) and/or the spinothalamic tracts (abnormality of temperature or pain sensation). The motor symptoms included weakness and sometimes

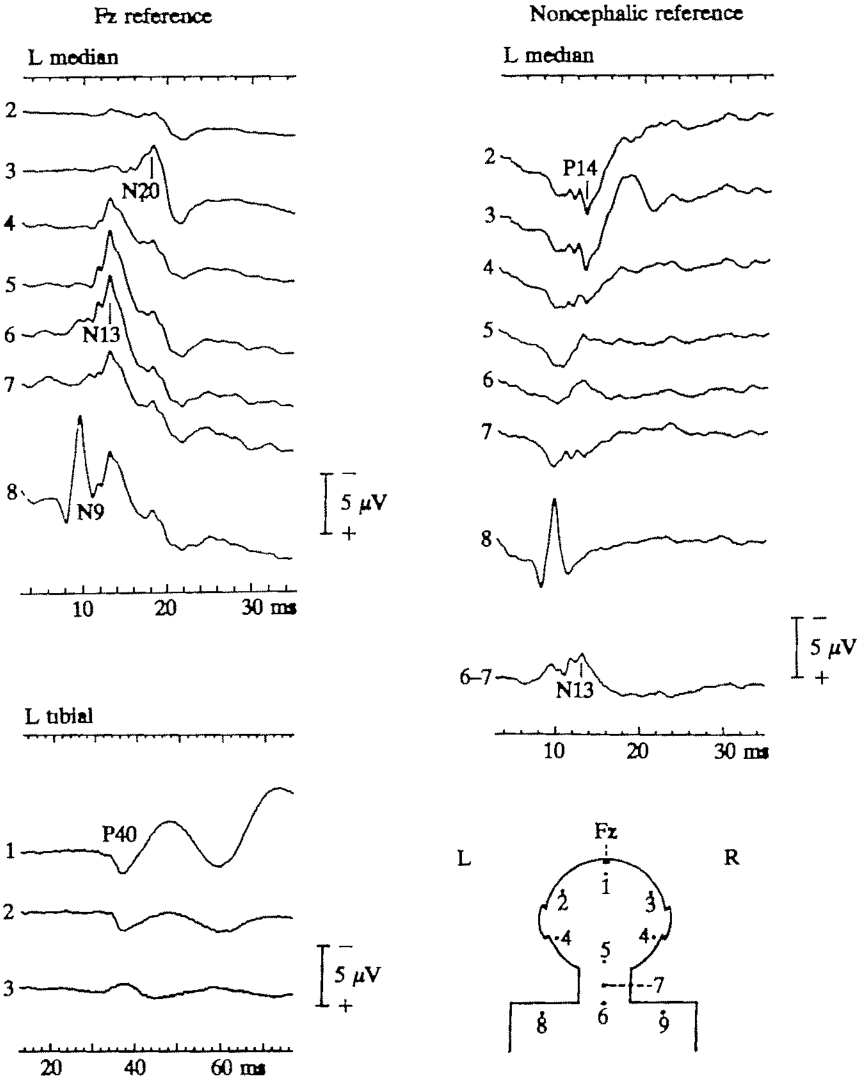

FIG. 1. Normal left median and tibial SEPs recorded over the foot cortical area (1), left and right hand cortical areas (2, 3), ipsilateral mastoid process (4), C2 (5), C7 (6), the anterior aspect of the neck on the midline above the thyroid cartilage ('supraglottal' location) (7) and the left and right clavicle (8, 9) with Fz reference The upper limb responses were also transformed off-line to noncephalic reference: channels 2–8 referred to clavicle (9) and channel 6 referred to channel 7.

stiffness with difficulty in walking; the motor signs were typical of lesions in the pyramidal pathway (spasticity, exaggerated tendon reflexes, extensor plantar reflexes, either decrease or absence of the abdominal reflexes). In the majority of cases sensory and motor deficits were asymmetrically distributed on the two sides. Clinical evidence of a lesion located in one side of the cord was found in 7 patients: 3 of these presented with the Brown-Séquard syndrome and 4 showed proprioceptive sensory loss in one hand, defined as the 'useless hand of Oppenheim'. Three patients of the latter group had also uni or bilateral motor deficits. Ten patients exhibited Lhermitte's sign and 10 had sphincter disturbance (Table 1).

SEP and MRI abnormalities

Cord and/or brain MRI was abnormal in 28 patients (90.3%), whereas SEPs were abnormal in 21 patients (67.7%). In the group comprising 27 cases of MS, 18 patients showed abnormal SEPs (66.7%), 18 from the median and 8 from the tibial nerve (in 2 patients the latter was not performed) and 25 showed abnormal MRI (92.6%), including 21 with cervical cord and 21 with brain lesions. In the group comprising 4 patients with isolated cord lesions, 3 showed abnormal SEPs (75%), 3 from the median and 2 from the tibial nerve (in 1 patient the latter was not performed), and 3 showed abnormal cord MRI (75%). In the Fz reference recordings, N13 and/or N20 components were abnormal in 18/31 cases (58.1%), whereas P40 was affected in 10/28 cases (35.7%). With the clavicle reference, P14 appeared abnormal in 14/31 patients (45.2%) and was ill-defined and so not interpretable in 4 cases. In the supraglottal reference waveforms, N13 was abnormal in 10/30 cases (33.3%), 2 of whom had normal Fz reference responses (Table 1). The most frequently observed abnormalities were decreased amplitude of N13 (Fz and supraglottal reference) and increased latency of N20, P40 and P14. N20 was absent in 1 case and P40 in 2 cases (fig. 2A).

MRI abnormalities of the cervical cord, affecting one or both sides, were of various sizes and extended from one to all cervical segments. In some cases the lesions were localized either anteriorly or posteriorly, whereas in others they involved both anterior and posterior regions. Similarly, in some cases the lesions were predominantly left or right-sided, whereas in others they were bilateral. In 5 cases the lesions were associated with swelling and in 3 cases with atrophy of the spinal cord (fig. 2B). Brain MRI lesions which it was considered might possibly involve the somatosensory pathways at supraspinal levels were observed in 20 patients. They were located bilaterally, unilaterally or centrally in the medulla (5 cases), pons (5 cases), internal capsule (3 cases), sensory radiations (17 cases) and sensory cortex (1 case). In 17 cases they were associated with MRI abnormalities of the cervical cord (Table 1).

Correlation between SEPs and MRI

SEPs and cord MRI were compared and the significance of correlations examined using the Fisher exact probabilities test.

In the Fz reference recordings from the median nerve there was a statistically significant correlation between N13 and/or N20 abnormalities and the presence of cervical cord MRI lesions ($P = 0.012$); this was demonstrated for left and right median nerve responses in relation to cervical cord lesions involving the left and right side, respectively ($P = 0.002$ and $P = 0.018$), and for median nerve responses from either arm in relation to lesions involving the posterior cervical cord ($P = 0.013$). Conversely, no significant correlation was observed comparing left and right median SEPs with right and left cervical cord lesions, respectively, or between median SEPs from either side and lesions involving the anterior cervical cord ($P > 0.05$). No significant overall correlation was observed comparing tibial or noncephalic reference median nerve SEP abnormalities with cervical cord lesions on MRI ($P > 0.05$) (Table 2).

TABLE 1. CLINICAL, SEP AND MRI ABNORMALITIES OF THE 31 PATIENTS INCLUDED IN THE STUDY

				Cord syndrome					*Median SEP*							
				Sensory		*Motor*		*Others*	*Left*				*Right*			
									Fz ref.		*NC ref.*		*Fz ref*		*NC ref.*	
Case no.	*Age (yrs)*	*Sex*	*Diagnosis*	*L*	*R*	*L*	*R*		*N13*	*N20*	*P14*	*N13n*	*N13*	*N20*	*P14*	*N13n*
1	27	F	CDMS	σ	σ	σφ	φ		*	*	*				*	
2	20	M	CDMS	σπ	σπ	σφ	φ	Lh Bl	*	*	*	*	*	*	*	
3	35	F	CDMS	σ	σ	σφ	σφ		*	*	*	*	*	*	*	
4	33	F	LSDMS	σπα	σπα	φ	φ	Lh					*			
5	38	M	LSDMS	σπ	σπ											
6	22	M	LSDMS	α	σα			Lh Bl								
7	31	F	LSDMS	σ		σφ	φ	Bl						*		
8	27	F	LSDMS	σα	σπα	φ	φ						*	*	*	
9	28	F	LSDMS	σ	σ			Lh				*	*	*	*	*
10	39	M	LSDMS	σα	σα	σφ		Bl								
11	36	F	LSDMS	σπα	σ	φ		Lh	*	*	*					
12	28	F	LSDMS	σ	σα	σ		Lh Bl					*	*	*	
13	26	F	LSDMS	σ	σ	σφ	φ									
14	36	F	LSDMS	σ		φ	φ	Bl								
15	50	F	CPMS			σφ	σφ						*	*	*	*
16	49	F	CPMS	σ	σ	σφ	φ	Lh								
17	40	F	CPMS			σφ	φ		*	*	NI	*	*	*	NI	
18	36	M	CPMS	π	π	σφ	σφ	Bl								
19	45	M	CPMS		α	σφ	σφ				*	*			*	*
20	26	F	CPMS	σ	σπ								*	*	*	
21	21	F	CPMS			σφ	φ					NR			NI	NR
22	25	F	CPMS		σπ		σ						*	*	*	
23	44	M	CPMS	σ	σ	σφ	σ	Bl	*		*					
24	48	F	CPMS	σ	σπα	σφ	σφ		*	*	NI		*	*	*	*
25	20	M	CPMS	σπ	σπ	φ	φ	Lh		*		*				
26	32	F	CPMS	α	σπ		σφ									
27	34	F	CPMS	σα	σα	φ	φ					*				
28	38	F	ICL	σ	σ	φ	φ			*			*	*		
29	38	M	ICL	σπα	σπα	σφ	σφ	Lh Bl								
30	39	F	ICL	σ	σπα	φ	φ						*	*	*	
31	26	M	ICL	σα	σα	φ	φ	Lh Bl			NI	*			NI	*

				Tibial SEP						Brain MRI lesions			
				Left	Right	Cervical cord MRI lesions				Somatosensory pathways			Other locations
				Fz ref									
Case no	Age (yrs)	Sex	Diagnosis	P40	P40	A/P	L/R	Levels	SW/AT	L	C	R	
1	27	F	CDMS		*	AP	R	2 3		Rad		Rad	*
2	20	M	CDMS	*	*	AP	LR	2 3 4 5 6	AT	Rad	Med		*
3	35	F	CDMS	*	*	AP	LR	4 5 6	SW	Int caps Rad		 Rad	*
4	33	F	LSDMS			P	LR	2		Rad	Med	Rad	*
5	38	M	LSDMS			AP	LR	3		Pons Rad		 Rad	*
6	22	M	LSDMS			AP	LR	3		Rad		Rad Int caps	*
7	31	F	LSDMS							Rad		Int caps	*
8	27	F	LSDMS	*	*	P	LR	2 3 4 5					
9	28	F	LSDMS			AP	R	5 6		Rad	Med		*
10	39	M	LSDMS			P	LR	3 5		Rad			
11	36	F	LSDMS			P	L	2 3			Med	Pons	*
12	28	F	LSDMS			P	R	1 2		Rad			*
13	26	F	LSDMS			P	LR	3		Rad		Rad	*
14	36	F	LSDMS			AP	LR	4 5	SW				
15	50	F	CPMS	*	*	AP	LR	1 2		Rad	Med	Rad	*
16	49	F	CPMS							Rad		Rad	*
17	40	F	CPMS	*	*	AP	LR	1 2 3 4 5 6 7		 Rad		Pons Rad	*
18	36	M	CPMS										
19	45	M	CPMS	*	*					Rad		Rad	*
20	26	F	CPMS			P	R	5				Cor	*
21	21	F	CPMS										
22	25	F	CPMS	*	*	P	R	CMJ			Pons	Rad	*
23	44	M	CPMS			A	LR	CMJ					
24	48	F	CPMS	NR	NR	P	LR	2 3	AT	Rad		Rad	*
25	20	M	CPMS	NR	NR	P	LR	2	SW				
26	32	F	CPMS			A	R	2 3	SW	Pons			*
27	34	F	CPMS										*
28	38	F	ICL	*	*	AP	LR	2 3	AT				
29	38	M	ICL										
30	39	F	ICL		*	A	R	CMJ					
31	26	M	ICL	NR	NR	A	L	2 3	SW				

Abbreviations. CDMS = clinically definite MS; LSDMS = laboratory supported definite MS; CPMS = clinically probable MS; ICL = isolated cord lesion; σ = sensory and motor symptoms; π = sensory signs implicating the posterior columns; α = sensory signs implicating the spinothalamic tract, ϕ = motor signs; Lh = Lhermitte's sign, Bl = abnormal control of the bladder; NC = noncephalic; N13n = N13 (supraglottal reference); * = abnormal, NI = not interpretable, NR = not recorded; A = anterior, P = posterior, L = left; R = right; CMJ = cervicomedullary junction; AT = atrophy; SW = swelling; C = central, Rad = radiation; Med = medulla; Int caps = internal capsule; Cor = cortex

Abnormalities of the latency and amplitude of N13 were assessed in relation to the levels of the cord lesions. The percentage of abnormalities of N13 recorded with supraglottal reference decreased in relation to the incidence of lesions at progressively higher levels, whereas the occurrence of abnormalities of N13 recorded with Fz reference was unrelated to the level of cord lesion (Table 3). Cord lesions located at C6 and C7 were significantly correlated with abnormalities of the supraglottal reference N13 ($P = 0.029$), but just failed to reach statistical significance with abnormalities of the Fz reference N13 ($P = 0.06$). When higher levels were examined, abnormalities of Fz reference N13 and clavicle reference P14 were significantly correlated with lesions located at C1 and/or the cervicomedullary junction ($P = 0.01$ and $P = 0.017$, respectively), whereas supraglottal reference N13 showed no such correlation.

The N9-N20 interpeak latency and the absolute latency of P40 were compared with the number of segments involved (i.e., the longitudinal extent of the lesion) on the ipsilateral side of the cervical cord. Both SEP measures were significantly correlated with the number of levels affected (left side $r = 0.57$ and 0.58, respectively, $P < 0.001$; right side $r = 0.38$ and 0.41, respectively, $P < 0.02$). For the tibial SEP the relationship between P40 latency and number of levels affected was approximately linear, equivalent to a delay of about 2 ms per level (fig. 3). However, since the variation in P40 latency was very wide for patients with lesions affecting 0, 1 or 2 levels and there were only 4 patients with longer lesions, too much weight should not be attached to this observation.

Using the Fisher test, no significant correlation could be demonstrated between abnormal SEPs from either side of the body and MRI lesions apparently involving

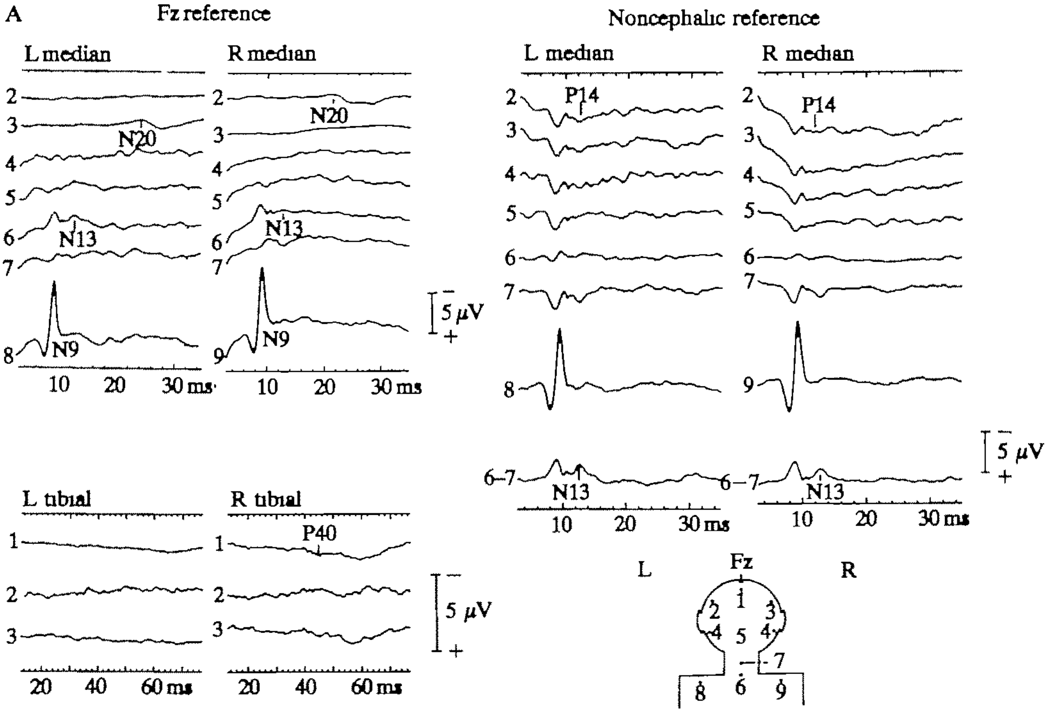

FIG. 2A.

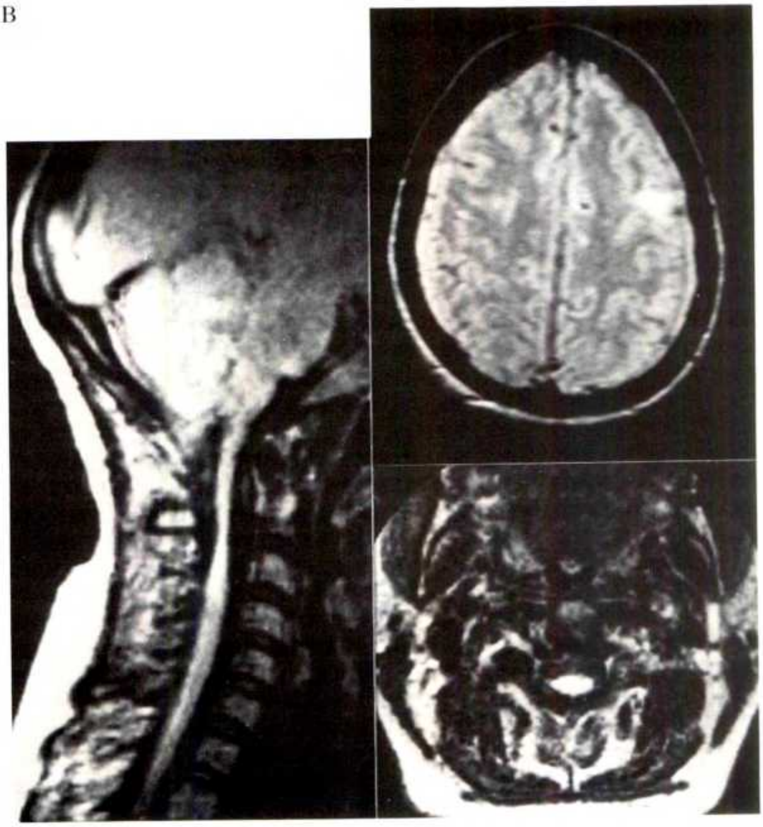

FIG. 2. *Case 28.* A, median SEPs recorded with Fz reference show bilaterally delayed N20 and degraded N13 on the right. From the tibial nerve, P40 is absent on the left and delayed and degraded on the right. In the noncephalic reference recordings, N13 is normal and P14, although not clearly defined, is within normal limits. B, *left*, sagittal view of the cervical cord, showing an area of increased signal at C2-C3 and marked atrophy at the same level. *Right lower*, axial view of the cervical cord, performed at the level of the lesion, showing anteroposterior flattening and increased signal throughout the cord. *Top*, axial view of the brain showing a left-sided lesion of the precentral cortex.

intracranial somatosensory pathways. Furthermore, the SEP abnormality involved the N13 component in 20/31 cases (27 arms), 17 of whom had ipsilateral MRI lesions in the cervical cord; 5 of the 17 also had lesions in the medulla, which might have contributed to the abnormality of N13, but the remaining 12 either had brain lesions which were located too far rostrally to affect N13 (6 cases) or had no brain lesions on MRI (6 cases). In 1 patient where the SEP abnormality involved only the cortically generated N20, it was possible that the defect could have been due to a lesion in the sensory radiations, visible on MRI.

Correlation between SEPs and cord syndromes

SEPs were abnormal in 17 out of 24 patients with sensory and motor impairment, in 2 out of 4 patients with only sensory abnormalities and in 2 out of 3 patients with isolated motor involvement. When SEPs were compared with sensory symptoms and signs separately, the latter showed a greater degree of correlation than the former,

TABLE 2. SIGNIFICANCE OF CORRELATION BETWEEN SEP AND MRI ABNORMALITIES*

	Median				
	Fz reference			*Noncephalic reference*	*Posterior tibial Fz reference*
	Both sides	*Left*	*Right*		
Cord	0.012			No correlation found	No correlation found
Left		0.002	n.s.		
Right		n.s.	0.018		
Post	0.013				
Ant.	n.s				
Brain	n.s.				
Left		n.s.	n.s.		
Right		n.s.	n.s		

* Fisher's exact probabilities test.

TABLE 3 PERCENTAGE OF N13 ABNORMALITIES IN RELATION TO THE LEVELS OF CORD LESIONS

		N13 abnormalities	
Cord levels		*Supraglottal reference*	*Fz reference*
C6–C7	(4)	100% (4)	100% (4)
C5–C4	(8)	50% (4)	75% (6)
C3–C2	(17)	35.3% (6)	58.8% (10)
C1-CMJ	(6)	33.3% (2)	100% (6)

No. of cases in parentheses CMJ = cervicomedullary junction.

although in neither case was this statistically significant. Thirteen patients had sensory signs consistent with lesions in the posterior columns and 13 patients in the spinothalamic tracts. The same incidence of SEP abnormalities was observed in both groups (69.2%).

Using the Fisher test, no significant correlation could be demonstrated between abnormal SEPs from either side of the body and ipsilateral or contralateral sensory symptoms, posterior column signs or spinothalamic tract signs, neither was there any correlation when these factors were combined into the broader category of 'sensory involvement'.

Correlation between MRI and cord syndromes

Cord MRI was abnormal in 24 patients, 18 with sensory and motor impairment, 4 only sensory and 2 only motor deficits. In some cases the cord lesion appeared smaller and in others larger than the lesion suspected on clinical grounds. Ten patients had an MRI lesion apparently involving only the posterior cord: 9 of them showed clinical sensory and motor abnormalities, whereas 1 complained only of sensory impairment. The 4 patients with only anterior cord MRI lesions showed both sensory (2 posterior column and 3 spinothalamic tract) and motor clinical impairment. Ten patients had a lesion involving the anterior and posterior cord: 5 of them showed sensory and motor, 3 only sensory and 2 only motor abnormalities.

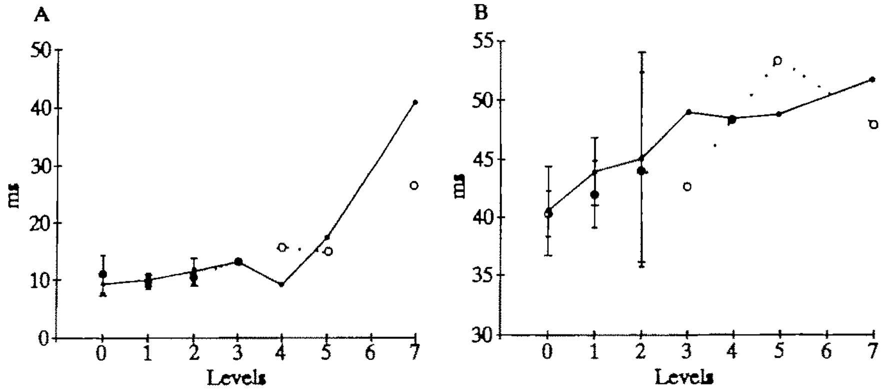

FIG. 3 Variation of N9-N20 interpeak latency (*left*) and P40 absolute latency (*right*) with the longitudinal extent of the cord lesion on the ipsilateral side. Continuous line = left side; dotted line = right side. Filled and empty circles = mean latency values for left and right side, respectively, vertical lines = their SDs. Only 4 patients showed cord lesions extending beyond 2 levels

The extent of cord MRI abnormalities was examined in relation to the distribution of sensory involvement. Twelve patients had bilateral cord MRI lesions and bilateral sensory impairment; 8 patients had unilateral cord lesions and bilateral sensory abnormalities; 1 patient had a bilateral cord lesion and unilateral sensory involvement; 1 patient had unilateral cord and ipsilateral sensory abnormalities. When cord MRI was compared with sensory symptoms and signs separately, the latter showed a higher degree of correlation than the former, although in neither case was this statistically significant, neither was any significant correlation demonstrated when sensory symptoms and signs were combined together. The upper level of the lesion in the cervical cord, as suggested by the distribution of sensory impairment, was in all but 1 patient at or below the upper level of the cord lesions seen in MRI, although it was not possible to establish a definite level from clinical data in every case.

No significant correlation was found between the extent of cord MRI abnormalities and the distribution of clinical motor involvement. Using the Fisher test, no significant correlation could be demonstated between sensory symptoms and/or signs on either side of the body and brain MRI lesions apparently involving the intracranial somatosensory pathways.

A relatively close association between clinical, electrophysiological and morphological data was observed in 4 patients presenting with the 'useless hand of Oppenheim' (Cases 11, 20, 22, 30; *see* Table 1). In all these cases, abnormal SEPs were evoked from the impaired arm. The abnormalities involved N13 and N20 in Fz reference and P14 in the clavicle reference recordings; the N13 component recorded with supraglottal reference was normal in each case. An abnormal P40 was recorded from both sides in 1 patient (Case 22) and from the clinically affected side in another (Case 30). MRI of the cervical cord showed in each patient an ipsilateral lesion involving the posterior side (Cases 11, 20, 22) or the anterior side (Case 30). The abnormalities were located at C5 level (Case 20), C2 and C3 levels (Case 11) and the cervicomedullary junction (Cases 22, 30). Three of these patients also showed brain lesions in the medulla, pons, sensory radiations and sensory cortex (fig. 4A, B).

TABLE 4. SIGNIFICANCE OF CORRELATION OF N13 AND P14 ABNORMALITIES WITH LEVELS OF THE CORD LESIONS*

Cord levels	*N13* *(Fz ref.)*	*N13* *(Supraglottal ref.)*	*P14* *(Noncephalic ref.)*
C1-CMJ	0.01	>0.4	0.017
C2-C3	>0.2	>0.3	>0.3
C4-C5	>0.1	>0.2	>0.2
C6-C7	0.06	0.029	>0.2

* Fisher's exact probabilities test

DISCUSSION

Abnormal SEPs are frequently recorded in patients with multiple sclerosis (Anziska *et al.*, 1978; Small *et al.*, 1978; Matthews and Small, 1979; de Weerd and Jonkman, 1982; Walsh *et al.*, 1982). Their incidence is higher in clinically definite than suspected cases, probably due to an increase in the total number of lesions with progression of the disease (Eisen *et al.*, 1979; Eisen and Odusote, 1980; Ganes, 1980; Green *et al.*, 1980; Kjaer, 1980; Khoshbin and Hallett, 1981; Matthews *et al.*, 1982). Abnormalities are usually obtained from limbs showing sensory impairment and also, although less frequently, from limbs in which no sensory deficits are present in the territory of the nerve tested (Namerow, 1968; Small *et al.*, 1978; Jones, 1982*b*; van Buggenhout *et al.*, 1982). MRI appears to be a more sensitive technique in detecting asymptomatic abnormalities in the brain in patients with MS, especially in the early stages of the disease

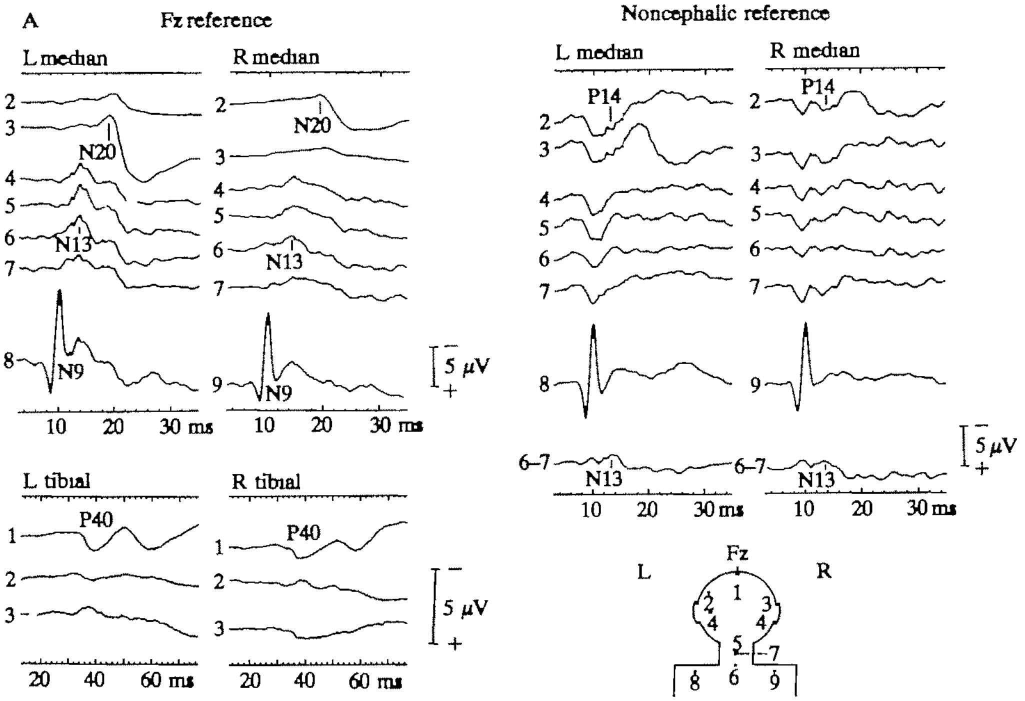

FIG 4A

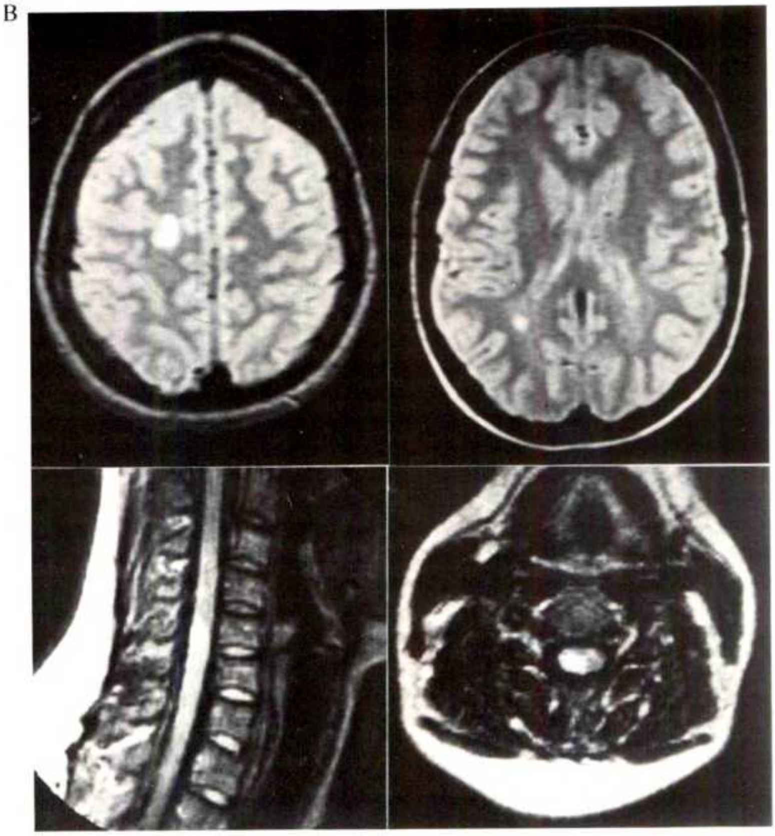

FIG. 4. *Case 20.* A, SEPs from the right median nerve show degraded N13 and slightly delayed N20 with Fz reference, and delayed P14 with clavicle reference. P40 from left and right tibial nerve is normal. B, *top*, axial views of the brain, showing areas of increased signal in the white matter of the right hemisphere. *Lower left*, sagittal view of the cervical cord, showing an area of increased signal at C5. *Lower right*, axial view of the cervical cord, performed at the level of the lesion, showing an area of increased signal in the right posterior quadrant.

(Miller *et al.*, 1987; Ormerod *et al.*, 1987; Paty *et al.*, 1988). Multifocal, asymmetric cerebral white matter lesions are seen in over 95% of patients with clinically definite MS (Young *et al.*, 1981; Lukes *et al.*, 1983; Runge *et al.*, 1984; Ormerod *et al.*, 1987).

The present study was performed in an attempt to come to a better understanding as to how morphological changes in the CNS shown by MRI correspond to electrophysiological changes in the somatosensory pathway detected by SEPs. We are aware of only one previous study (Matthews and Esiri, 1979) in which SEP abnormalities have been related to a plaque in the cervical cord, discovered at autopsy. We also wished to determine the degree of correlation between the abnormalities demonstrated by these techniques and specific clinical deficits.

It was found that abnormalities of N13 and/or N20, recorded after median nerve stimulation with a reference at Fz, were significantly correlated with MRI abnormalities located in the posterior half of the cervical cord (and hence probably involving the posterior columns) or on the ipsilateral side, but not in the anterior half or on the contralateral side. Abnormalities of P40 following tibial nerve stimulation were less well correlated with MRI lesions involving the posterior or ipsilateral hemicord. The correlation between the length of the MRI abnormality in the ipsilateral hemicord and the N9-N20 interpeak latency or the absolute latency of P40 was significant, although the value of this observation was limited by the fact that only 4 patients showed lesions extending beyond two levels. These results provide evidence that MRI abnormalities involving the posterior half of the cervical cord are with high probability associated with electrophysiological abnormalities of sensory conduction. That there is a correlation of the longitudinal extent of MRI cord lesions with the amount of SEP delay suggests that demyelination is a factor in these lesions.

In the present study abnormal responses occurred less frequently from the tibial (35.7% of cases) as compared with the median nerve (67.7%). Conversely, previous studies (Chiappa, 1980; Trojaborg *et al.*, 1981; Bartel *et al.*, 1983; Rossini *et al.*, 1985) reported that in patients with MS the incidence of abnormalities is usually higher in the SEPs from the lower limbs, in accordance with the fact that the investigation of a longer segment of the somatosensory pathway increases the possibility of detecting abnormalities. One explanation for this discrepancy could be that the patients examined in this study were not representative of the MS population, having been selected for cervical cord involvement and without evidence of more caudal spinal cord lesions. All the patients with abnormal tibial nerve SEPs also had abnormal median nerve responses, hence a lesion at cervical level could have been responsible for both abnormalities. In the cervical cord, sensory structures related to the upper limbs occupy a greater volume than those associated with the lower limbs. For this reason, in patients with cervical cord lesions, abnormal SEPs from the upper limbs are likely to be more frequent than abnormal lower limb responses. Nevertheless, the finding of abnormal lower limb SEPs associated with normal median nerve responses might possibly occur due to a small lesion affecting the gracile tract only.

It was found that abnormalities of N13, recorded at C7 with a supraglottal reference, correlated well with MRI lesions located at the lower cervical levels, whereas abnormalities of P14, recorded from the scalp with a clavicle reference, correlated best with MRI lesions involving C1 and the cervicomedullary junction. These findings are in agreement with the general view that the N13 component is mainly generated by activation of interneurons in the grey matter of the dorsal horn, at levels where the dorsal roots from the upper limbs enter the spinal cord (Desmedt and Cheron, 1981), whereas the P14 component corresponds to the advancing front of propagating activity in the somatosensory pathways at or above the foramen magnum (Mauguière *et al.*, 1983; Mauguière and Ibañez, 1985).

With the methods used in the present study, it seems unlikely that brain MRI abnormalities are frequently responsible for the electrophysiological deficits detected by SEPs. This result is perhaps not surprising if we consider that the localization of MRI abnormalities in specific pathways of the brain can only be made with some approximation. Also most of the brain MRI lesions (85% in this study), which usually appear small and disseminated, are located in periventricular areas, where the fibres arising from the somatosensory nuclei of the thalamus diverge in their projection to various areas of the cortex. For this reason, it is possible that the fibres activated by median or tibial nerve stimulation may not have been involved in the MRI lesions, or that the damaged proportion was insufficient to cause electrophysiological changes detectable by SEPs. Furthermore, demyelination is not necessarily present in all MRI lesions in MS. Oedema (which increases mobile water proton density and hence alters MRI signal) alone produces MRI abnormalities (Barnes *et al.*, 1987), and experimental and MRI/pathological studies have shown that abnormal MRI signals in demyelinating diseases are generated by lesions in which there are proportions of inflammation, oedema, gliosis and demyelinaton, changing with time (Barnes *et al.*, 1988; McDonald and Barnes, 1989; D. Barnes, D. G. MacManus and W. I. McDonald, unpublished observations). Demyelination per se produces little change in MRI signal (Bottomley *et al.*, 1984). These findings provide a possible explanation for the fact that SEPs appear sometimes to survive the existence of extensive lesions in the sensory radiations or the spinal cord (fig. 5A, B).

A previous study (Eisen *et al.*, 1987), carried out in possible and probable MS cases, showed a significant correlation of cortical median and tibial SEP abnormalities with MRI lesions involving the somatosensory pathways and mainly located in the periventricular areas. The most frequent SEP abnormality reported was distorted morphology of the cortical response, detected by a fast Fourier transform technique (Roberts *et al.*, 1983) or a method of dynamic time warping (Eisen *et al.*, 1986). The recording of cortical SEPs with restrictive (high band-pass) digital filtering was also used by Rossini *et al.* (1985) to detect high-frequency subcomponents normally contained within the latency range of N20 and P40. Abnormalities of these wavelets were reported in 2 suspected MS cases, in whom the cortical response recorded with open band-pass filtering appeared normal. The present study cannot confirm or refute these findings, although personal observation suggests that these high-frequency subcomponents may not be present even in some normal subjects.

In the full group of 31 patients no significant correlation was found between SEPs and sensory abnormalities on either side of the body. Abnormal SEPs were recorded from limbs with or without sensory deficits and no specific correlation could be observed with symptoms or impairment of particular sensory modalities. The same incidence of abnormalities was obtained from limbs showing impaired 'spinothalamic' (temperature and pain) or 'posterior column' (joint position, two-point discrimination, vibration) type sensation, although previous studies of MS and other conditions (e.g., Halliday and Wakefield, 1963; Yu and Jones, 1985) suggested a stronger correlation with the 'posterior column' modality. This finding may be partly explained by the fact that in this study components recorded with both cephalic and noncephalic reference were examined, whereas in previous investigations only the former were usually considered.

In the full group of 31 patients there was also no significant correlation between cervical cord MRI abnormalities, in axial and sagittal views, and the pattern of sensory or motor impairment. There are some possible explanations for such a discrepancy. First, the resolution of spinal MRI is still significantly limited. Interpretation of spinal images is impaired by surface coil inhomogeneity and CSF motion artefacts (Miller *et al.*, 1987). Also the limit of resolution to a voxel size of 15 mm^3 (pixel size 1.2 mm×2.4 mm, slice thickness 5 mm) makes it likely that small lesions were not seen; MS plaques smaller than 15 mm^3 are seen at postmortem (Fog, 1950; Oppenheimer, 1978). Furthermore, localization of a lesion to a quadrant of the cord does not inevitably mean that a particular tract is involved, as has been implicitly assumed in this study. Despite all these factors, it was observed that in patients with sensory deficits, the upper limit of the cervical cord abnormalities seen in sagittal views was consistently at or above the level suggested by the clinical examination. Therefore, it appears that spinal MRI often identifies the symptomatic pathological process, although not with a precise anatomical-functional correlation.

A greater degree of correspondence between electrophysiological, morphological and clinical abnormalities was found in 4 patients presenting with the 'useless hand of Oppenheim'. This is characterized clinically by a profound loss of joint position sense in one upper limb, largely sparing the lower limb, which has generally been attributed to a lesion in the cuneate tract. Abnormal SEPs were recorded from the affected arm in all 4 cases and an abnormal lower limb response in 2 cases; MRI showed in each case a lesion located in the appropriate side of the cervical cord between C5 and the cervicomedullary junction. The clear correlations in these 4 patients probably reflect the precise localization possible on clinical grounds: more often, demyelinating cord syndromes suggest a patchy, ill-defined lesion. These findings are in keeping with those in other syndromes, in which similar precise localization is possible: acute unilateral optic neuritis and deafness in MS (Barratt *et al.*, 1988; Miller *et al.*, 1988).

In conclusion, the present study has shown that in patients with demyelinating cervical cord lesions, the morphological changes seen in MRI of the cord are usually associated with appropriate electrophysiological abnormalities. On the other hand, the 'clinically silent' MRI lesions in the brain seldom, if ever, give rise to SEP abnormalities, using standard methods. Both SEP and MRI abnormalities correlate to some extent but not

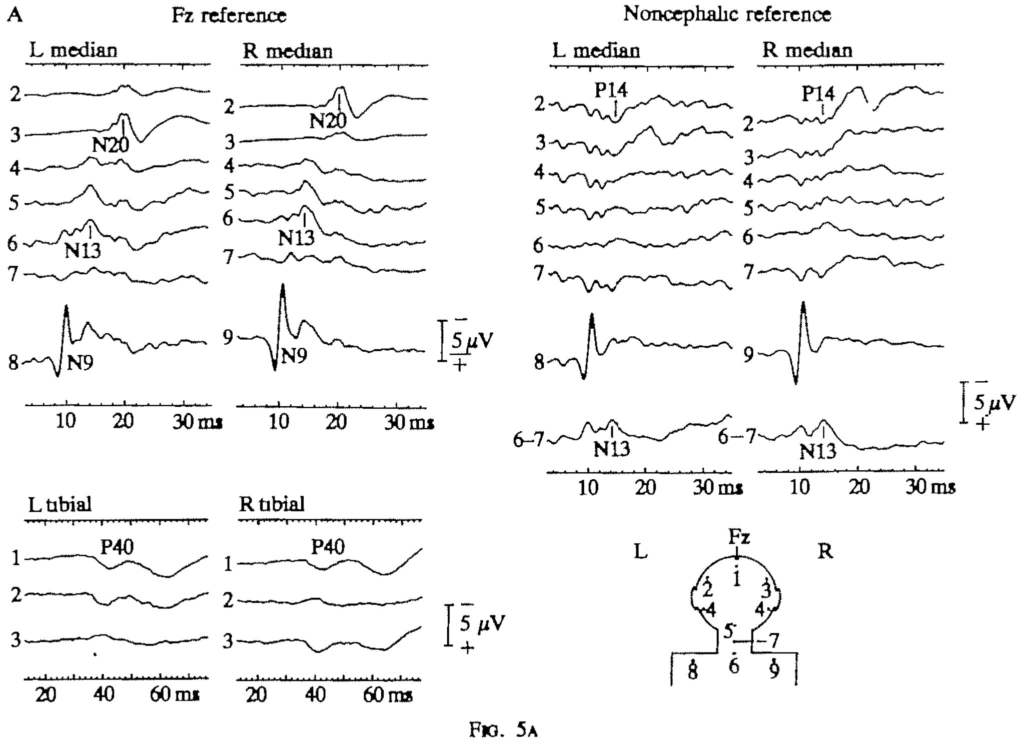

FIG. 5A

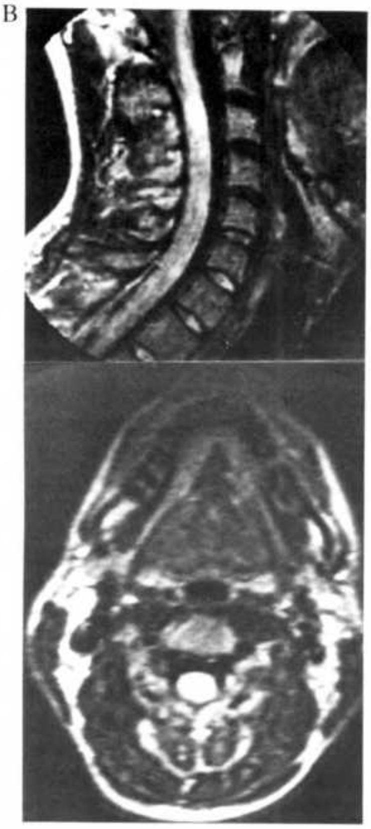

FIG. 5. *Case 6.* A, normal median and posterior tibial SEPs. B, *top*, sagittal view of the cervical cord, showing an area of increased signal at C3 level. *Bottom*, axial view, performed at the level of the lesion, showing an area of increased signal involving the whole cross-section of the cord.

perfectly with the pattern of clinical deficits. This could be partially due to the approximation introduced by the subjective evaluation of the clinical findings, the fact that SEPs provide only an incomplete measure of conduction in sensory pathways, and the likelihood that regions of abnormal signal seen in MRI are not always representative of or coterminous with an area of myelin damage.

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(Received December 28, 1989. Revised April 6, 1990. Accepted April 26, 1990)

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BOOK REVIEWS

Trigeminal Neuralgia. Edited by R. L. Rovit, R. Murali and P. J. Janetta. 1990. Pp. 288. Baltimore: Williams and Wilkins. Price £70.00.

The editors of this well presented book have collated 13 chapters which review virtually all aspects of trigeminal neuralgia and its management. The individual authors are acknowledged experts in the field and discuss frankly the technical aspects of those interventional procedures that they advocate.

An excellent historical review by Robert Wilkins is followed by detailed summaries of anatomy and physiology, clinical features and medical management. Murali reviews the frequently ignored practice of peripheral nerve injection and avulsion, advising rightly that it still be considered in the infirm. The percutaneous procedures of radiofrequency thermocoagulation, balloon compression and glycerol injection are described lucidly and in sufficient detail by Rovit, Mullen and Lunsford to encourage even the most ardent proponent of any one technique to consider the others. Rhoton, renowned for his work on microsurgical anatomy, presents a beautifully illustrated chapter demonstrating the simplicities and complexities of the trigeminal nerve and its surgical relations while Janetta (synonymous with microvascular decompression) presents the results of over 700 procedures. No account of trigeminal neuralgia would be complete without considering the distress of anaesthesia dolorosa; Nashold writes convincingly of the nucleus caudalis DREZ procedure for this complication of surgery. Apfelbaum compares the relative merits of percutaneous and microvascular procedures from his experience in over 1000 patients. Sweet, the doyen of neurosurgical pain relief, analyses the world literature and comments on the results and complications of surgical intervention.

Those faced either regularly or infrequently with patients suffering from tic doloureux will find their questions answered in this book. It will be of interest to all neurologists and neurosurgeons, no matter which regime they advocate, that painful postoperative dysaesthesiae occur with almost the same incidence following each procedure even when performed by an expert. Although it is unlikely that any individual will change his chosen procedure after reading these pearls of wisdom, all will benefit from reading the observations of this well chosen group of authors within one cover. At its publication price *Trigeminal Neuralgia* is a worthy and informative addition to the library of any neurological and neurosurgical department and to the shelves of individuals.

Colin Shieff

Chronic Epilepsy, its Prognosis and Management. Edited by Michael R. Trimble. 1989. Pp. 260. Chichester: John Wiley. Price £38.50.

Considering the complexity and uncertainties inherent in the management of patients with intractable epilepsy, Michael Trimble has done an excellent job in keeping this book down to just over 250 pages. Some 23 authors contribute to 18 chapters which are in general clear and concise, reflecting careful and strict editing. Each chapter carries an extensive reference list.

The pathophysiology of chronic epilepsy (Meldrum) is given only a relatively small amount of space, but is covered in sufficient detail for most clinicians. Excellent and authoritative sections on electrophysiological investigation (C. D. Binnie) and surgical treatment (C E. Polkey), are justifiably allowed more space than some others. The editor contributes two lucid reviews on difficult fields relating to cognitive and behavioural consequences of chronic epilepsy but, sadly, little advice on the management of these problems is offered. A rather didactic chapter on the use of antiepileptic drugs (Duncan) is followed by two short sections on the value and limitations of adjunctive agents including benzodiazepines.

Although it is relatively short, this book covers a large number of overlapping fields encountered in the care of children and adults with chronic epilepsy. It is eminently readable and very well referenced, and will be valuable to clinicians involved in the day-to-day care of people with epilepsy, both as an up-to-date review and a source of key publications in the field.

R. E. Cull

Neurobiology of Incontinence. Ciba Foundation Symposium 151. Edited by Greg Bock and Julie Whelan. 1990. Pp. 336. Chichester: John Wiley. Price £35.95.

This book was compiled according to the usual Ciba Foundation formula: a symposium arranged, speakers and discussants invited, and the text of the talks and taped discussion subsequently published as a book. The advantages of this method of producing a textbook must be considerable. The invited speakers for this symposium were all leaders in their fields and therefore heavily committed academics or doctors but the condition of the symposium required that they provide texts so that within 9 months of the meeting (no doubt a busy time for the editorial team at Ciba) the book was published. No waiting for that last overdue chapter! Also, but for the symposium it is unlikely any single editor could have persuaded so many eminent names to write for a single title.

The topics discussed were all germane to the problems of incontinence, both urinary and rectal. Burnstock spoke on the innervation of the bladder and bowel, de Groat on the central neural control of the lower urinary tract. Gynaecologists spoke on aspects of stress incontinence, urologists on neurogenic bladder disorders and rectal surgeons on rectal incontinence, means of examining rectal physiology and operative procedures for improving rectal incontinence. Pharmacologists discussed medical treatments of urinary incontinence and Brindley reviewed the results of using his implanted root stimulators. Swash, who must be congratulated on his suggestion of the topic to the Ciba Foundation, talked about a neurogenic hypothesis of stress incontinence. The written chapters from these talks are mostly excellent and the references are a valuable source of current work.

The most fascinating aspect of the book is the discussions. Participants are discouraged from burnishing their comments excessively when sent the verbatim transcripts of the discussion to check, and the close questioning and unrehearsed answers are there to be read. Ex cathedra statements were not allowed to pass without critical comment and several points of fundamental disagreement were aired. Also, facts that it might have been assumed were 'well worked out' are revealed as being not understood by the experts; detrusor instability was perhaps the most striking example of this. There is no discernible difference between idiopathic and neurogenic detrusor instability and smooth muscle physiologists think the problem may even be myogenic.

The book has been nicely printed and the illustrations have come out well. It is very reasonably priced and would be valuable for anyone who wished to read a contemporary review of the subject by way of a general introduction, as well as for the reader who knows the field and has views about the controversies which were discussed. My only regret about this book is that somehow no indication comes across as to the pleasure of the meeting. This is a pity because it was a most enjoyable symposium.

CLARE J. FOWLER

Genetics and Alzheimer's Disease. Edited by P. M. Sinet, Y. Amour and Y. Christen. *Research and Perspectives in Alzheimer's Disease.* 1989. Pp. 179. Heidelberg: Springer. Price DM 108.00.

This new addition to the excellent Springer-Verlag series on Research and Perspectives in Alzheimer's Disease is based on the proceedings of the meeting on Genetics and Alzheimer's Disease held in Paris in March 1988. The contents of the book range widely within this expanding field with the majority of the major groups working in the field being represented. The editing is excellent and the familiar faults in such books—the repetition of historical introductions with each chapter and inconsistencies between authors—have been avoided. The preface is helpful, giving a synopsis of the most important data; it also updates the reader on developments between the conference and publication.

Over 2 years have passed since the conference but although some of the work is now dated much remains relevant. In particular, the 3 chapters from the neurogenetics laboratories of Massachusetts General Hospital, Duke Medical Center and Antwerp concerning progress in the search for the Alzheimer's gene (or genes) give a useful appraisal of the progress made and the problems encountered. These problems have still not been entirely resolved.

Progress has been made rapidly in the last 2 years in other areas particularly in the study of the amyloid protein precursor gene, its expression and posttranslational modifications. However, the chapters in this book dealing with amyloid β-protein precursor protein remain an excellent introduction to the more recent work. There is a useful review of research on the structure of the paired helical filaments which are prominent in the neurofibrillary tangles found in neurons in Alzheimer's disease. Other areas covered, although more briefly, include work on animal models of Down's syndrome and early results from Epstein's work on transgenic mice in Alzheimer's disease.

There are also 'nonmolecular' contributions. There is an account of the IMAGE project centred in Quebec Province, Canada. This project aims to ascertain all cases of Alzheimer's disease within the area, using this region as a 'geographical laboratory' to study the disease. The multidisciplinary approach of this North American group has the potential to lead to major advances in our understanding of the aetiology of Alzheimer's disease in the longer term.

One of the more interesting papers by Rapoport contains the hypothesis that Alzheimer's disease is a phylogenetically new disease—that it does not affect nonhuman primates or other mammals. He also suggests that elucidation of the mechanisms of Alzheimer's disease may lead to advances in our understanding of the evolution of higher brain functions.

Other chapters deal with further aspects of this disease including dermatoglyphic patterns in Alzheimer's disease, the epidemiology of Alzheimer's disease and the problems involved in tracing extended kindreds in familial cases.

This book should be required reading for scientists involved in Alzheimer's research; there is also much to interest clinicians who care for patients with this devastating disease. Hopefully, there will be future volumes detailing progress using the newer molecular techniques such as the polymerase chain reaction and transgenic animals.

JEREMY BROWN

Acquired Speech and Language Disorders. A Neuroanatomical and Functional Neurological Approach.
By B. E. Murdoch. 1990. Pp. 349. London: Chapman and Hall. Price £14.95.

Speech and language disorders are rarely covered in detail in any of the standard neurological texts, and specialist monographs, e.g., Benson's *Aphasia, Alexia and Agraphia*, now 11 years old, are few. This title advertises 'A neuroanatomical and functional neurological approach', a text which aims to bridge the gap between neurology, anatomy and linguistic theory. Neuroanatomy and neurology are described in each chapter and are followed by a detailed discussion of the speech pathology literature relevant to the area with the aim of linking the two.

The professional origins of the writer are not identified though presently he is head of the Department of Speech and Hearing at the University of Queensland, Australia. The preface attributes him with two decades of teaching in the areas of neuroanatomy and acquired speech language disorders to speech pathology students but it is not stated whether he is neurologically qualified. In the text there are enough small errors of concept or fact to suggest that the writer does not have hands-on experience of clinical neurology. This may be irritating to clinicians and raises anxieties about the rest of the book. Examples include comments in a paragraph on head injury, about subarachnoid haemorrhage resulting from the rupture of an aneurysm the assignment of different types of aphasia to different vascular pathologies without any mention of lacunar disease, and an impression that sodium MR is as available as an imaging technique.

Speech pathology sections seem much better and are well referenced. Discussions about Bostonian and Lurian schools of aphasia include mention of the origins of these different approaches. There are sections on subcortical and right hemisphere language disorders, dementia, dysarthrias and acquired aphasias of childhood.

Neurologists have by and large been slower than their speech pathology colleagues in recognizing the role played by subcortical structures and the right hemisphere in the grammatic and prosodic content of language; the author has amply reviewed a fascinating growth area. The section on apraxias revives the 1908 discussion of ideomotor, ideational and limb kinetic apraxia with no mention of present concepts of higher order movement disorders. Constructional and drawing apraxias, which many would classify as being disorders of space, find their way into this section. The author seems on happier ground when discussing apraxia of speech. There is the expected diversion into alexias and agraphia in which the brief review of frontal and deep alexia is useful. The chapter concludes with a limited review of the agnosias; the pedant would speak of Anton's syndrome rather than disease. The discussion of Gerstmann's syndrome places it firmly as part of the angular gyrus syndrome.

The chapters concerned with dysarthria, including extrapyramidal, hyperkinetic and ataxic dysarthria also contain clinical oddities; an odd definition of fasciculation, mention of the grasp reflex to differentiate upper from lower motor neuron lesions, referring to ataxia as a condition. The author's classification of the motor dysarthrias is not fully justified, and dysphonia is not mentioned other than in a discussion on the vagus nerve. The unduly detailed division of brainstem vascular disorders into their eponymous syndromes seems old-fashioned.

There is an impressive list of symptoms and signs attributable to cerebellar disorders; does anybody record asthenia these days? The differential diagnosis of cerebellar disorders excludes alcohol and the paraneoplastic syndromes. Friedreich's ataxia is not, in my practice, the most common degenerative cause of ataxia and does ataxia occur in 20–30% of myxoedematous patients? It is a pity there is no discussion of the effect of disorders of the control of ventilation on speech production other than its mention under multiple sclerosis.

The final chapter concerns acquired language disorders in children. It begins with a list of causes of acquired cerebral insults which includes the electroencephalogram! The speech pathology section of the chapter is of interest to the adult neurologist and reminds one that children are not just little adults.

At no point in the book is there any mention of the place played by the speech therapist; perhaps the author is unconvinced they have any therapeutic role in that he does not cite any controlled clinical trials in support of their work.

Not only would this book have fulfilled its purpose with better editing, perhaps by a neurologically trained second author, but it also needs a simple glossary of speech science or linguistic terms. The grammatically illiterate need to be reminded of pragmatics, semantics, spirontization and deviant speech dimensions to make this book more of an interface between disciplines. I regret that this book will not be read by neurologists and I would be hesitant to recommend it to other clinicians or medical students. Speech pathology students might find the speech science useful but should learn their neurology from a standard text. It's a shame; the idea is a good one and the second edition, with careful editing, might actually live up to the expectations of the preface.

GRAHAM VENABLES

Hemispheric Specialization and Psychological Function. By J. L. Bradshaw. 1989. Pp. 218 Chichester: John Wiley. Price £26.95.

Bradshaw's book is an update of Bradshaw and Nettleton's *Human Cerebral Asymmetry* in a more popular style and more extensively illustrated. It is a review, but it also has elements of a textbook with references to other reviews and to key articles, rather than referencing all the statements It is similar to several other recently published volumes (most of them edited) in its content, but manages to be up-to-date, comprehensive yet concise, and unique in its individual style and interpretation of the data.

The book starts with asymmetries in nonliving organisms and proceeds to birds, rodents, primate and human asymmetries. The review of animal functional laterality studies are particularly well done. The next section on clinical studies of human cerebral asymmetry is a practical synopsis of recent advances in neuropsychology and it appears to be well integrated. The diagram illustrating the language-related areas is a little too schematic at the expense of accuracy. The labelling of Wernicke's area is an error (inferior instead of superior). The review provides equal exposure to well established taxonomies, as well as to more recent iconoclastic views, that try to get rid of group studies and classifications. Nonverbal syndromes also receive attention; in particular face recognition and neglect are extensively reviewed. The commissurotomy chapter is quite sophisticated. At the end it waxes philosophical, discusses the issues of consciousness, and the duality of the mind. The chapter on normal studies not only discusses the technical aspects of laterality experiments, but also summarizes the rather large literature in the field. This is information that neuropsychologists working not in this particular area will welcome in a summary format.

A considerable amount of integration is achieved in the chapter on models of hemisphere interaction. Following this, a few individual chapters appear to be more specialized and stand by themselves. Language and the right hemisphere is comprehensive. The section on the anthropology of language development and tool use stands somewhat alone, but is undoubtedly relevant to hemisphere lateralization. Handedness and sex differences are useful summaries of some of the theoretical controversies and experimental evidence in these areas. There is a brief chapter on the ontogeny of hemisphere lateralization, and finally an overview of hemisphericity and cognition sounds the appropriate amount of caution to attribute individual differences to hemisphericity.

The style of the book is lively, at times conversational, with a rather continuous flow of words, interrupted by questions and a lot of explanations in brackets. It is readable, but not for the beginner. Students with a high level of motivation and attention will benefit a great deal. Peers will find it a useful addition to their library.

ANDREW KERTESZ

Tourette Syndrome and Human Behaviour. By David E. Comings. 1990. Pp. 828. Duarte, CA: Hope Press. Price $49.95.

The Gilles de la Tourette syndrome has attracted considerable attention in recent years. It was first described in 1885 by the man after whom it is now called. It was of sufficient interest to Charcot for him to recognize the condition as unique enough for it to be given the eponymous title 'Gilles de la Tourette syndrome' Interestingly the literature on this syndrome then dwindled, only to be resurrected in the 1970s, largely by the Shapiros. Since that time a considerable number of publications have been devoted to the subject, and more recently several books have appeared. Of all of these, that by Comings is perhaps the most original. It is written 'for both the lay reader and the health or education professional' and is said to be a guide to the 'exciting story of how a common gene may control a wide range of human behaviours'. Comings is a geneticist, and has been one amongst several who have explored relationships between differing phenotypes in this condition. Thus it is now accepted that the Gilles de la Tourette syndrome is not purely a tic disorder, but one which involves multiple differing behaviours ranging from chronic motor tics, through complex motor behaviours, to more obvious psychiatric syndromes such as obsessive compulsive disorder.

Much of the recent literature is reviewed in this book which has a bibliography of over 2 100 references and covers all aspects that may touch upon the syndrome. These include excursions into neuroanatomy, neurochemistry, genetics, and a wide range of behaviours which a number of authors would consider irrelevant. Thus it is the view of the group from Duarte that Gilles de la Tourette syndrome is a hereditary disorder which has a wide range of expressions which include everything from attention deficit-hyperactivity disorder, dyslexia and learning disabilities to mood swings, depression, alcoholism and drug abuse, overeating, phobias, panic attacks and even migraine. This view derives from their own studies, well documented here, which include the giving of their human behaviour questionnaire to nearly 250 patients. A copy of the questionnaire is included as an appendix. Their preferred neuroanatomical location for the functional deficit in the syndrome is in the limbic system, and their preferred culprit neurotransmitter is serotonin.

Certainly there is plenty in this book for those who wish to find out about the Gilles de la Tourette syndrome. It is well illustrated, and explains many issues clearly, which will be of great help to nonmedical personnel dealing with the syndrome who use the book. Physicians may be a little irritated by the simplicity of some of the text, and also by the naivety of some of the theories. However, as a useful source book on the Gilles de la Tourette syndrome, this can be highly recommended.

MICHAEL TRIMBLE

Surgery of Spine Trauma. Edited by Paul E. Meyer. 1989. Pp. 867. New York and Edinburgh: Churchill Livingstone. Price £99.50.

Traditionally, neurosurgeons have been concerned with the neurological consequences of spinal disease, leaving their orthopaedic colleagues to manage the skeletal problems. All this is changing and neurosurgeons are now beginning to address themselves to the problems of spinal instability, whether these are caused by trauma or disease. At the same time new ranges of devices for internal fixation of the spine have been developed and these have been widely used by both specialties.

This present monumental volume was written by an orthopaedic surgeon and is concerned with the management of the skeletal aspects of spinal trauma. It contains no discussion of many of those facets of spinal injury which are of most concern to the neurologist and neurosurgeon, such as the acute treatment of neurological damage, posttraumatic syringomyelia or the control of posttraumatic pain and sphincter disturbance. Although over two-thirds of the book is devoted to fractures of the spine and the internal and external means of stabilizing them, the preliminary chapters provide thorough accounts of the initial treatment of spinal injuries, their anatomical basis and their radiological investigation. The use of evoked potentials and the management of associated chest and head injuries are also covered. Nevertheless, despite the limited scope of this book it is strongly recommended to every neurosurgeon with a large spinal practice who should know something of what his orthopaedic colleagues are undertaking, some of which he should perhaps attempt himself. This book is exhaustive, authoritative and well written, quite apart from being well produced and well illustrated. It is supplied with a useful bibliography. It should become the standard work in its field.

R. S. MAURICE-WILLIAMS

Neuronal Grafting and Alzheimer's Disease. Edited by F. Gage, A. Privat and Y. Christen. *Research and Perspectives in Alzheimer's Disease.* 1989. Pp. 202. Berlin: Springer. Price DM 108.00.

This book represents the proceedings of a symposium organized by the Fondation Ipsen in September 1988. The book is splendidly produced with some colour illustrations, each chapter standing alone and representing a proper review of the subject, rather than a simple brief summary of a piece of work. The book is thus unusually well produced and unusually well edited. Neuronal grafting is clearly an experimental area of major current importance and, although the book is principally concerned with the possibility of neuronal grafting for the repair and compensation for cognitive defects in Alzheimer's dementia the initial 5 chapters are concerned with more general aspects. Many readers will find the book worth purchasing for these 5 chapters alone. The first, by the editors, provides an introductory review of the subject of neuronal grafting, identifying some of the key issues. The following chapter by Björklund and colleagues considers the subject of nigral and striatal grafting to the experimentally lesioned neostriatum as a model of graft function. Five different possible levels of function following grafting are identified, namely; non-specific consequences of transplantation, as pharmacological action of the graft by secretion of biologically active molecules, tonic reinnervation, unregulated by dynamic change, reciprocal reinnervation in which the graft is partially under the control of host nervous system and, ultimately, full reconstruction with re-establishment of functional connections. The evidence that intrastriatal grafts can establish extensive reciprocal connections with the brain is reviewed as are the other possible mechanisms for functional effect in a chapter that should be a model for those struggling to understand the potential of this new technique. The fate of intracerebral grafts of dopaminergic neurons is reviewed in Chapter 3 by Herman and colleagues and there follow 2 fascinating chapters, one on Purkinje cell replacement in an animal model of heredodegenerative ataxia and a second on spontaneous remyelination and intracerebral grafting of myelinating cells in mammals.

The remainder of the book is concerned more specifically with the problem of the possibility of neural transplantation in Alzheimer's disease. In these 12 chapters a variety of ingenious approaches to this problem are identified and considered in detail, in relation not only to the identification of suitable animal models but, more centrally, in experiments to ameliorate the effects of ageing in the rat, and in the methodology of physiological monitoring of the functional role of putative grafts. This is a timely and well-produced book that is a model of the value of symposium proceedings, when well focused, and well edited.

MICHAEL SWASH

Clinical Magnetic Resonance Imaging. Edited by Robert Edelman and John Hesselink. 1990. Pp. 1192. Philadelphia: W. B. Saunders. Price £117.95.

This is a multiauthor textbook dealing comprehensively with Magnetic Resonance Imaging. About one-third of the text is devoted to a complete review of the physics and instrumentation. Very sensibly the fundamentals of the subject are covered in two chapters and these are set out in simple terms, very suitable for students with no prior knowledge of the subject Although the remainder of the physics section is much more advanced and extends to cover all aspects of the subject, it is still explained in terms which can be understood by general medical readers and this is further aided by the use of excellent diagrams.

Another third of the book is devoted to the central nervous system. This is covered regionally and subdivided under pathological processes in conventional fashion. The pathophysiology, clinical manifestations, significant features on CT and other imaging modalities are briefly reviewed in relation to the interpretation of the MR features and differential diagnosis. Though this section is at a level suitable for radiologists with an interest in magnetic resonance imaging, it should prove of considerable interest to neurologists and neurosurgeons.

The remainder of the book deals with MRI of the chest, abdomen, pelvis and musculoskeletal system and there are appendices dealing with scanning protocols and a glossary of magnetic resonance terminology.

The book is well produced, the images are of excellent quality, the references are up-to-date and there is a good index. It is an excellent reference book for radiologists including neuroradiologists and particularly for those specializing in magnetic resonance imaging. The initial chapters are recommended to clinicians seeking an introduction to magnetic resonance and the section on the nervous system is very appropriate for clinical reference.

BRIAN KENDALL

Imaging of the Nervous System. Edited by Paul Butler. 1990. Pp. 334. Berlin: Springer. Price DM 348.00.

I am continually being stumped by postgraduate students of neurosciences or radiology asking me to recommend a single volume textbook of neuroradiology, so the immediate question posed by this book is: will it put me out of my predicament? The answer is an unequivocal yes . . . and no.

Why the rider? Mainly because the authors (13 British imaging specialists, mainly young) or editors of this book (Dr Butler) or the series *Clinical Medicine and the Nervous System* (John Conomy and Michael Swash) appear to have taken a conscious decision to exclude not only interventional neuroradiology but also almost all discussion of normal radiological anatomy, techniques, indications, complications, cost benefit considerations, etc., in favour of a straightforward review of pathological appearances. This is evidently a defensible approach, particularly if the book is aimed at radiologists, who should learn about those other aspects as part of their general training. One could, however, argue that the things omitted are exactly those of which neurological physicians and surgeons need to be aware, and that they might leave interpretation of images to their colleagues in the imaging department.

Within its chosen terms of reference the book generally succeeds, although the balance is not always right: in 1990 anything other than a passing comment on conventional cerebral scintigraphy would seem excessive, particularly when paediatric ultrasound is confined to two images of the head, and none of the spine. The inclusion of a detailed discussion of contrast media is questionable. The form of the individual chapters is variable and some read like texts of clinical pathology with radiological asides. A number of the contributors seem obsessed by statistics and many include questionable statements transferred verbatim from the literature, which I should have expected their own experience to have led them to modify or even reject. The quality of the illustrations varies from excellent to acceptable, while the index is merely adequate.

What shall I say to the postgraduate students? I shall recommend this book, with the reservations expressed here, and suspect that it will prove very successful, although the price (approximately £100.00)—to which a single page of coloured SPECT images may have contributed disproportionately—could limit sales.

IVAN MOSELEY

Atlas of Electroencephalography. By Alan Guberman and Madeleine Couture. 1989. Pp. 216. London: Little, Brown. Price £110.00.

This book consists almost exclusively of illustrations of samples of EEG records. The reproductions are generally of high standard but inevitably have been recorded with different makes and models of apparatus over a period of some years. Electrode placement is uniformly in accordance with the well-known 'International (10–20) electrode placement' system and this system is presented for reference as part of a very brief introduction to the use of electroencephalography in clinical practice. At the end of the volume there is an index of illustrations and also a useful list of references and a bibliography. The illustrations are all of good quality and show approximately 20 s excerpts from 16 or 17 channels. The sample pages are reduced during the reproduction process by about 50% which gives a good compromise between the ability to see fine detail in the records and a reasonably convenient format. The pages are spiral bound so that they lie flat in use and the whole is protected by a hard cover.

The contents are divided into 'chapters' which cover the normal EEG, awake and asleep, physiological and technical artefacts, normal and abnormal responses to photic stimulation, epilepsy with examples of ictal and interictal patterns, status epilepticus, coma, 'nonspecific patterns' and a miscellaneous section of EEGs in 'various neurological and medical conditions'.

This book will be invaluable for teaching EEG departments and as a source of illustrations for use by fairly experienced workers in the field. It will be of much less value to individuals, whether neurologists with an interest in EEG or students, because of the absence of any accompanying text; arrows indicating important phenomena are insufficient for the naïve reader. This deficiency is to some extent made up for by the fact that each illustration or sequence of illustrations has a brief descriptive heading including an indication of the clinical diagnosis and an indication of the relevant references to be consulted. For example, we are shown an excerpt from an EEG showing 'Interictal generalised atypical spike-wave bursts (2–2½ Hz)'. We are told that the record is taken from an 18-year-old male with Lennox-Gastaut syndrome and we are referred to papers by Blume *et al.* in *Brain*, 1973, Gastaut *et al.* in *Epilepsia*, 1966 and Markand in *Neurology*, 1977. Not all illustrations have associated references, however, for example a rather similar illustration on the facing page of an excerpt of EEG from a patient with familial myoclonus epilepsy.

A particularly useful feature of this atlas is that where the context requires an excerpt of EEG longer than 20 s, for example when the evolution of seizure discharges is illustrated, then a series of pages consecutive or more or less consecutive in time is used.

In summary, then, this is a fairly comprehensive set of 275 illustrations of sections of EEG records with useful references which should be invaluable in any teaching centre and well worth the asking price of £110.

H. R. A. Townsend

Neuromonitoring in Surgery. *Clinical Neurophysiology Updates*, Volume 1. Edited by J. E. Desmedt. 1989. Pp. 377. Amsterdam: Elsevier. Price Dfl. 295.00.

All right-minded surgeons are agreed as to the desirability of monitoring the nervous system during risky procedures, and most of the rest are resigned to the prospect that in some circumstances the failure to monitor will be considered negligent. Amongst clinical neurophysiologists, surgical monitoring is recognized as a major responsibility for the future; even the least strongly committed would admit that this is a worthy, if unexciting, field for medical advance. It is indicative of the high level of current interest that the present volume comes first in a series of *Clinical Neurophysiology Updates*, and follows at least two substantial monographs and two books of proceedings which have appeared in the last 5 years.

After 5 introductory chapters we find a collection of 15 articles from authors well chosen to represent the status of intraoperative monitoring in their various specialties. Each provides a detailed account of his or her own experience, but only a minority review the published literature in any depth. What the volume lacks, which perhaps reflects the immaturity and fragmentation of the field, is an overall perspective of what can be done with the available techniques, what are their relative merits and failings and what are the major problems to be addressed in the future. There is no discussion of 'cost effectiveness', which causes no difficulty for me although it may limit transatlantic sales. Seriously lacking is an anaesthetist's perspective, since not only do pharmacological factors have a great bearing on the size and variability of evoked cortical potentials, but many anaesthetists have been at the forefront of neuromonitoring developments and their colleagues are likely to have to cope with these techniques in addition to their traditional responsibilities. Other areas regrettably unrepresented are electrocorticography and intraoperative EEG monitoring, and the use of SEPs for identifying sensory and motor areas of the exposed cortex.

The first 2 chapters review the fundamentals of somatosensory and brainstem auditory evoked potentials in perhaps too much detail for those interested only in their surgical applications. At this point one might expect to find an overview of the use of these techniques at operation and the effects of anaesthetic agents, but Chapter 3 describes a personal approach to the problem of improving the signal/noise ratio, combining a system of 'phase synchronized triggering' (which might more accurately be termed 'phase desynchronized' and is one of the most elementary of methods for reducing mains-frequency noise) with a two-dimensional filtering technique which looks impressive on paper but lacks combat experience. Chapters 4 and 5 cover, with some duplication, the techniques and consequences of motor cortical stimulation by electricity and electromagnetism, and on page 89 we at last find a description of practical experience with the former in monitoring efferent pathways during spinal surgery.

The rest of the book is genuinely surgical in tone, ranging from monitoring the action of muscle relaxants (the only contribution from an anaesthetic department), through techniques for monitoring orthopaedic spinal, posterior fossa and cerebral vascular procedures, to extra-surgical monitoring of epileptic seizures using epidural electrodes and the use of SEPs to assist with the implantation of stimulating electrodes for pain relief. Most if not all contributions recycle material published elsewhere, but it is good to find, for example, chapters from the Mayo Clinic covering a wide range of peripheral and central monitoring applications alongside the more specialized experience of groups from the USA, Japan and Europe. I suppose it is too much also to expect exciting reading and major revelations—after all the ideal of monitoring is to have a totally uneventful operation. Judging from the series title, the book is aimed mainly at clinical neurophysiologists, but can also be commended to anaesthetists and surgeons who are concerned with these issues. It can be considered a worthwhile document of the current state of about 75% of the field, and would be more recommendable at 50% of the price.

S J. JONES

The Clinical Relevance of Kindling. Edited by Bolwig and Michael Trimble. 1989. Pp. 302. Chichester: John Wiley. Price £40.00.

This book is based upon a symposium held in Helsingør, Copenhagen, in November 1988. The book is divided into 17 chapters dealing with different aspects of the kindling phenomena and also includes 6 sections or summaries of the relevant discussions which took place at the meeting.

There is now an extensive literature on the kindling phenomena and a number of previous books dedicated to the subject. This book contains a mixture of short papers each by different authors, many which attempt to relate different medical phenomena to the kindling process. The multiauthor nature of the book has led to a degree of redundancy with several chapters going over similar introductory ground in regard to the initial descriptions of kindling phenomena. The ground covered in the book is quite extensive. For example, the possible application of a kindling model is not only considered in relation to several problems of epileptology such as the prognosis of epilepsy and the development of new drugs, but also in relation to a variety of psychiatric fields including panic attacks, schizophrenia, anxiety, drug withdrawal and other addictive behaviours. While it is interesting to see such a diverse and at times speculative set of papers placed together, it did give the reader the impression that the kindling model was being used in such a general sense as to detract from its value. People working within one discipline using the kindling model to explain particular phenomenology may find it interesting to see how other disciplines have manipulated this model, although readers without a specific interest may be confused or at least not helped by this amalgamation of work.

D. R. FISH

Perspectives in Memory Research. Edited by Michael S. Gazzaniga. 1988. Pp. 330. Cambridge, MA: MIT Press. No price given.

'Anyone with even a passing professional interest in memory has been faced with the smiling face of a relative probing, ". . . you study memory? . . . I have a terrible memory . . . can't remember names. How can you help me?" Most students of memory return the smile, mumble something about things being complex, and scan the room for a more pleasant conversationist, for the truth is that despite many years of study little is known about how to improve memory' (Hirst and Gazzaniga, p. 275).

The present book seeks to review current knowledge about both the neurobiology and the cognitive psychology of memory to see how close we are to improving the situation. Gazzaniga is best known for his work on hemisphere specialization and, as an overview of the topic, the present book contains a somewhat unusual collection of authors as well as a slightly surprising mixture of topics. For example, there is a chapter on imagery in learning, which is fair enough, but begs the question why there is not a corresponding chapter on language and memory. The early chapters are on the neurobiology of memory—a chapter on the biochemistry of information storage by Black and colleagues, a chapter on structure-function relationships by Lynch and Baudry, a chapter postulating a 'basic circuit' of memory organization in the cortex by Shepherd, and one on connectionist models of memory by Sejnowski and Rosenberg. There follow 3 chapters which are supposed to be on 'psychological dimensions' of memory function, although the first of these is in fact a review of event-related potential studies (Kutas). There are then chapters on 'improving memory' (Hirst) and on 'imagery in learning' (Kosslyn), followed by an overall review of trends by Hirst and Gazzaniga.

The early chapters tended to be packed with detail, a little repetitive, and not very readable. The later chapters gave a rather patchy account of the psychology and neuropsychology of memory, although they were good in parts, and Hirst in particular had some interesting things to say about strategies for memory retraining. However, the most striking feature of the book was that it illustrated the enormous gulf between our understanding of the neurobiology of memory and the problems which the psychology of memory poses. Black, Lynch, Baudry, Shepherd, and Sejnowski and Rosenberg only scratch the surface in terms of providing an account of the phenomena which Hirst and Kosslyn describe.

In their summing-up, Hirst and Gazzaniga suggest two ways, and reject a third, by which this gulf may be bridged First, they note the importance which Lynch and Baudry attribute to the hippocampus in the encoding of contextual information. Since Hirst himself has argued, on the basis of neuropsychological studies, that impaired encoding of contextual information is a major deficit in patients who have organic amnesia, Hirst and Gazzaniga see this issue as being one where neurobiological and psychological studies are pointing in the same direction. Secondly, the authors see 'connectionist models' as a powerful means of bringing together neurobiological and psychological studies (in a way first envisaged by Hebb 40 years ago). These models postulate networks of connected nodes which can show impressive learning if the weights of the connections are allowed to change with experience, and may mimic the workings of neurons and synapses. However, the authors correctly emphasize several limitations of connectionist theory. Hirst and Gazzaniga are much more dismissive of neurochemical and psychopharmacological studies, claiming that 'acetylcholine . . . is investigated simply because people with memory problems have an impoverished level in the brain, not because it is known to have anything to do with memory'. I would disagree with this profoundly, arguing that not only is it depleted in various neuropsychiatric disorders, but that the pattern of anterograde memory impairment produced by cholinergic blockade closely resembles that seen in organic amnesia.

In summary, it has to be said that elsewhere there are better places to learn about the neurobiology of memory (e.g., Squire's *Memory and Brain*, 1987) and better reviews of the psychology of memory (e.g., Baddeley's *Human Memory—Theory and Practice*, 1990). Nevertheless there are certainly things of interest in the present book. Moreover, a statement by Hirst and Gazzaniga (p. 298)—'Some of the mnemonic processes that supply the glue that holds individual events together and creates a unified representation are lost with amnesia . . '—demonstrates, perhaps inadvertently, just how far we have to go in coming to grips with this topic.

MICHAEL KOPELMAN

Neuromethods, 13. Psychopharmacology. Edited by Alan A. Boulton, Glen B. Baker and Andrew J. Greenshaw. 1989. Pp. 821. Clifton, NJ: Humana Press. Price £100.40.

The first thing to notice about this book is the price: £100.40. The second thing to note is that, although I had expected a detailed discussion of studies of the cognitive, behavioural and psychiatric effects of neuroleptics, anticholinergic agents, antidepressants and benzodiazepines, the 821 pages of this book are virtually all confined to consideration of research in animals. Thirdly (and probably related to the second point), when I looked up 'placebo effect' in the index, nothing was to be found.

The book discusses such topics as drugs and motor asymmetries, nociceptive responses, feeding and drinking, explorative and operant behaviour, spatial preference, electrical brain stimulation, discrimination and other learning, drug tolerance and ethopharmacology. However, in the absence of any consideration of pertinent studies in humans, such discussion constitutes a sterile enterprise. Animal research is, of course, a vital component for the development of new pharmacological agents but, when its findings have been taken in isolation, they have often proved to be misleading. For example, animal studies of vasopressin suggested it played a specific role in memory processes, but much of the research in humans has failed to support this (Kopelman and Lishman, 1986) and now the animal researchers themselves are changing their minds. Secondly, studies of the cognitive effects of anticholinergic agents in humans provide a broadly consistent picture (Kopelman, 1986), but when these results are pooled with findings from animal studies (Collerton, 1986) there are many conflicts which are almost impossible to interpret. A third topic, in which it seems confounding results are likely to be obtained between human and animal studies, is the use of 'brain transplants' as neurotransmitter replacement therapy: though dramatic successes have been obtained in animals, early findings in diseased humans suggest that the results are going to be very disappointing.

Some concession to these points is made in the chapter by Franklin and Abbott, who write 'despite the sensitivity of the tail-flick and hot-plate tests to the mu-agonist opioids, the threshold for pricking pain in human subjects is not sensitive to clinically used doses of these drugs . . .' They proceed to consider the 'tail-immersion test and its variants', the tail-pinch test, and the inflamed paw pressure test, all likely to be of limited value in humans. More promising are the flinch-jump test, the tooth-pulp stimulation test, the shock-induced vocalization test, the writhing test, and the shock-titration test, but the names of such procedures make one wonder if the animal liberationists have a point.

In short, the present book adds to my growing belief that human cognitive psychology and applied animal behaviour often amount to separate disciplines covering superficially similar topics. The present book has virtually nothing to say to neurologists, clinical pharmacologists, cognitive psychologists, or psychiatrists, and would more appropriately be entitled 'Neuromethods in Animal Drug Studies'. Those who wish to persist in this narrow discipline may find £100 worth of value here, but those researchers interested in clinical problems and applications would be better advised to invest elsewhere.

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Michael D. Kopelman

Cerebral Cortex. *Development and Maturation of the Cerebral Cortex.* Volume 7 Edited by A. Peters and E. G. Jones. 1988. Pp. 518. New York: Plenum Press. Price $75.00.

This series provides a collection of authoritative essays about the state-of-the-art research on the cerebral cortex. Previous volumes have discussed the structure and connectivity of the mature cerebral cortex and the functions of neurons and glia. Volume 7 contains 13 chapters on the development of the mammalian, including the human, neocortex and hippocampus. Neurogenesis, cell migration, morphological and functional development of fibre projections and local circuits, neurotransmitter and synaptic maturation and plasticity, vascular ontogeny, together with environmental influences including nutrition are all discussed by an international team of high calibre scientists. The use of axon tracer techniques and immunohistochemistry have revolutionized neurobiological research and much of this new material relevant to cortical development is presented so that each article offers a balanced appraisal of present day concepts. In concentrating on classical developmental neuroanatomy and neurochemistry, the one deficiency of this volume is that the impact of molecular biology on modern cortical research has been largely neglected. There are a couple of chapters which give a taste of this exciting development. However, in the one on cytoskeletal proteins, biochemical parameters are the major theme, and in the other, on the reeler mouse mutation, structural defects are documented, with little consideration of either the genetics or the molecular aspects. Each chapter has a comprehensive reference list which is an extremely useful source of most of the literature on cortical development.

Martin Berry

Elements of Molecular Neurobiology. By C. U. M. Smith. 1989. Pp. 525. Chichester: John Wiley. Price £17.95.

Few will argue that molecular biology has the potential to enhance our understanding of many neurological disorders. As the bandwagon (gravy-train?) proceeds, the vocabulary expands, the mystique increases and mistrust sets in. It is important, therefore, that the principles of molecular biology can be presented in an accessible and interesting way. This book is a triumph of clarity of thought, expression and presentation. It seeks to introduce the reader gently to the vocabulary, methodology and application of molecular biology. In this task, it is supremely successful.

The book begins with a summary of the structure and function of DNA, RNA and proteins before moving on to explain how current techniques enable analysis of the human genome. This section explains the use of cloning, the creation and use of libraries, and sequencing. The reader is then taken through chapters on biophysics, bioenergetics and neurophysiology. There is a useful section on second messengers. The final sections of the book are devoted to the molecular basis of brain function including growth cones, cell adhesion molecules and plasticity, before discussing 'five pathologies', three of which are neurodegenerative disorders. Each chapter is concise and yet informative.

Where will this book find its audiences amongst the medical fraternity? It might take pride of place in the library of a medical student intent on having a broad understanding of the use and usefulness of molecular biology. It will be kept on the littered desk of the medical researcher new to the area of molecular biology. He or she will find this book invaluable as a means to revise his/her basic biochemistry and to become familiar with current techniques and their application. Working neurologists will keep this book at the bedside (their only place for books of leisure). Those unfamiliar with the subject will be able to use the book to help them understand the techniques contributing to the explosion of new information regarding neurological disorders. Finally, a few copies may find their way into the libraries of neuroscientists, academic neurologists, etc. Here the book may be secreted away, but in a place readily available for quick reference to fill in embarrassing gaps in knowledge (e.g., gene strutting).

This book is not as comprehensive as for instance *Molecular Cell Biology* by Darnell, Lowdish and Baltimore, or *Biochemistry* by Lehninger. However, these books are intended more as reference sources rather than as an introductory text. As an enjoyable and informative read, C. U. M. Smith's book is highly recommended.

ANTHONY SCHAPIRA

ANNOUNCEMENTS

Ninth International Congress of Electromyography and Clinical Neurophysiology

This Congress which will be held in Jerusalem, Israel, has been postponed to May 1992. It is planned for the week beginning May 24. Further information may be obtained from Dr A. N. Gilai, PO Box 29334, Tel Aviv 61292, Israel.

International Quincke Symposium

This symposium, organized under the auspices of the World Federation of Neurology, will be devoted to Barrier Concepts and Cerebrospinal Fluid Analysis. It will be held in Göttingen, on September 19–21, 1991. For information, contact Professor K. Felgenhauer, Neurologische Universitätsklinik, Robert-Koch-Strasse 40, D-3400 Göttingen, FRG.

British Aphasiology Society

A conference will be held at Sheffield University on September 19–21, 1991. Abstracts of papers not exceeding 150 words with an accompanying summary of not more than 500 words (2 copies of each) should be sent, by March 31, 1991, to Dr Graham Venables, Department of Neurology, The Royal Hallamshire Hospital, Sheffield S10 2TN.

Euromyasthenia III

The Third European Conference on Myasthenia Gravis will be held at Wadham College, Oxford, UK on July 3–5, 1991. Further information may be obtained from Dr A. Vincent, Neurosciences Group, Institute of Molecular Medicine, John Radcliffe Hospital, Headington, Oxford OX3 9DU, UK.

25th Meeting of the Canadian Congress of Neurological Sciences

This will be held at the Halifax Sheraton, Halifax, Nova Scotia, on June 19–22, 1991. For information, contact Permanent Secretariat, Canadian Congress of Neurological Sciences, 810, 906 12 Avenue, SW, Calgary, Alberta, Canada T2R 1K7 (403) 229–9544.

3rd Meeting of the International Neurotoxicology Association

This will be held in Salsomaggiore Terme, Parma, Italy on July 1–5, 1991. Topics to be covered will include subcellular and cellular mechanisms of neurotoxicity, neurotoxicity and ageing, developmental neurotoxicity and screening for neurotoxicity in humans. For information, contact Dr A. Mutti, Organizing Secretary 3rd INA Meeting, Laboratory of Industrial Toxicology, University of Parma, Via Gramsci 14, I-43100 Parma, Italy Telefax +39-521-29 13 43.

2nd International Congress on Evaluation Scales in Rehabilitation Medicine

This will be held in Montreal, Canada, on October 4–5, 1992. The principal theme will be the reliability of methods of evaluation, especially as applied to vertebral dysfunction and head trauma. For information, contact Pierrette Boivin, 6300 Darlington Street, Montreal, Quebec, Canada H3S 2J4. Telephone (514) 340-2089.

Society for Research into Hydrocephalus and Spina Bifida

The 35th Annual Scientific Meeting will be held in Liverpool, England, on July 10–13, 1991 For details, contact Dr. R. Bayston, Institute of Child Health, 30 Guilford Street, London WC1N 1EH. Telephone 071-242-9789

3rd World Congress on Sleep Apnea and Rhonchopathy

This will be held in Tokyo on September 21–23, 1991 Details may be obtained from the Secretariat for the Third World Congress on Sleep Apnea and Rhonchopathy (III SCSAR), Simul International, Inc., Kowa Building, No 9, 1-8-10 Akasaka, Minato-ku, Tokyo 107, Japan. Telephone 81-3-3586-8691.

European Neurological Society

The third meeting will be held at the Palais de Beaulieu, Lausanne, Switzerland on June 27–July 1, 1992. The deadline for the receipt of abstracts is January 15, 1992. Further information may be obtained from Professor Andreas Steck, Service de Neurologie, Centre Hospitalier Universitaire Vaudois, 1011 Lausanne, Switzerland.

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ISSN 0006-8950

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Brain (ISSN 0006-8950) is published bi-monthly by Oxford University Press, Southfield Road, Eynsham, Oxford OX8 1JJ, England. Annual subscription price in USA $195.00 including air speed delivery. Second class postage paid at Jamaica, NY 11431.

USA POSTMASTERS: Send address changes to *Brain*, Publications Expediting, Inc, 200 Meacham Avenue, Elmont, NY 11003. Air freight and Mailing in the USA by Publications Expediting, Inc, 200 Meacham Avenue, Elmont, NY 11003.

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1 Mumford JP Br J Clin Pract 1988, 42 (Suppl 61) 7-9

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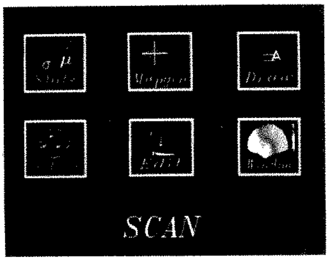

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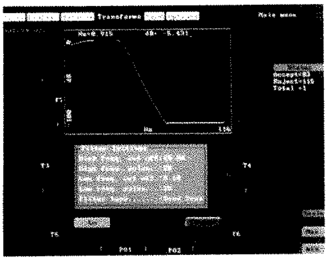

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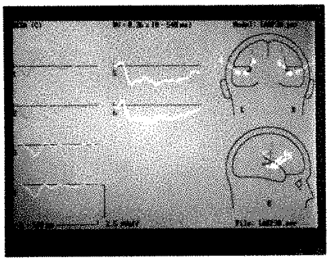

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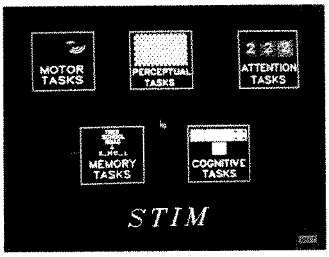

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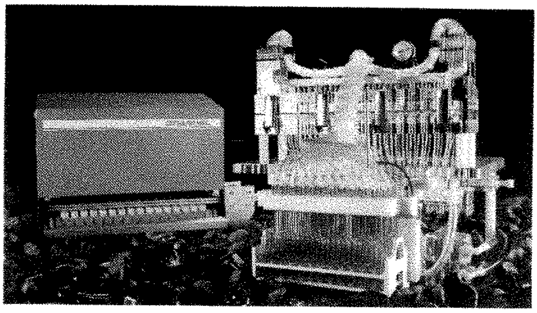

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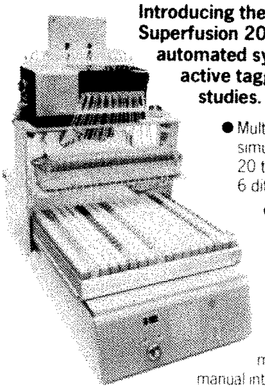

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A JOURNAL OF NEUROLOGY

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MODIFICATIONS OF THERMOREGULATION IN PATIENTS WITH SUPRASELLAR PITUITARY ADENOMAS

by R. BEHR,[1] G. HILDEBRANDT,[1] M. KOCA[1] *and* K. BRÜCK[2]

(*From the Departments of* [1]*Neurosurgery and* [2]*Physiology, Justus-Liebig University, Giessen, Germany*)

SUMMARY

Thermoregulation was investigated pre and postoperatively in 5 and only preoperatively in 7 patients with suprasellar pituitary adenomas by exposing them to external cold and heat in a climatic chamber. Five healthy subjects served as controls. Body core and skin temperatures, oxygen consumption, electromyographic activity, skin blood flow and local sweating rates were continuously measured. Threshold temperatures for activation of heat production and heat loss were calculated from these data. Hormone analysis was performed before and after stimulation with releasing factors. In the patients, core temperatures as well as threshold temperatures for heat production and heat loss were elevated by about 0.5° C as compared with controls. This elevation of core and threshold temperatures was achieved, despite a reduced resting metabolic rate, by a reduction of skin blood flow indicated by a low mean skin temperature. After successful operation the thermoregulatory alterations normalized. Serum levels of growth hormone were reduced preoperatively and stimulation by GHRF did not cause an appropriate increase. Prolactin was elevated in 6 patients with prolactinomas, but there was no correlation with changes in thermoregulatory threshold temperatures. Stimulation of the other hypophyseal hormones by the combined anterior pituitary function test revealed a normal hormonal response. Apart from prolactin there were no significant hormonal changes postoperatively. It is concluded that disturbances of temperature regulation are present in patients with suprasellar adenomas, but that they are not detectable by routine clinical methods. These alterations probably depend on a disturbance of hypothalamic function and are reversible by surgery.

INTRODUCTION

Changes in body temperature may occur in patients with hypothalamic disorders (Strauss and Globus, 1931; Davison and Selby, 1935; Alpers, 1936; Globus and Kuhlenbeck, 1942; Cooper, 1970; Fox *et al.*, 1970). Before spontaneous alterations in body temperature become manifest, there could be a stage, especially with slowly growing tumours, when thermoregulatory disturbances exist but are not detectable by routine temperature measurement. The aim of this study was to investigate such alterations of thermoregulation in patients with pituitary adenomas extending above the chiasmatic plane and compressing the hypothalamus. For this purpose the patients were exposed to external cold and heat in a climatic chamber and the reactions of thermoregulatory effectors measured in relation to changes in deep body and mean skin temperature.

METHODS

The patients and healthy control subjects were exposed to cold and heat in a climatic chamber, dressed in bathing clothes and resting in a supine position in a chair. After an initial period of 30 min at an ambient

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temperature (T_a) of 30° C (wall and air temperature equal; relative humidity 50%), T_a was reduced to 10° C until the patients and controls exhibited shivering, indicated by recordings of electromyographic (EMG) activity and increased metabolic rate (MR). The cooling period lasted for 40 min from the start, when it was 30° C, to the lowest ambient temperature. The subjects were then rewarmed by increasing T_a to 55° C (relative humidity 50%). This heat exposure was continued until heat dissipation reactions (vasodilation, sweating) occurred, which happened at about 80 min after the warming period had been started.

The following variables were measured continuously and recorded by an electronic data acquisition and processing system.

Temperature

All temperatures were measured using copper-constantan thermocouples.

Air temperature (dry bulb temperature). This was recorded in the middle of the climatic chamber (T_a). Air movement was less than $0.5\ \mathrm{m \cdot s^{-1}}$. The thermoprobe was insulated for thermal damping.

Head core temperature. Temperature in the external auditory canal close to the tympanic membrane—tympanic temperature (T_{ty})—was taken as the head core temperature. The auditory canal was plugged with absorbent cotton and the ear was covered by a capsule filled with absorbent cotton. This site of measurement was used as some of the patients were intolerant of an oesophageal temperature probe, especially after transnasal operations. There is no major difference between oesophageal and tympanic temperature under stationary conditions, as demonstrated in previous studies in this laboratory (Hessemer and Brück, 1985).

Skin temperatures. This was measured at 4 different sites: upper arm, chest, thigh, lower leg. Mean skin temperature ($\bar{T}_{sk}$) was determined using the equation of Ramanathan (1964).

Mean weighted body temperature. The thermoregulatory responses are influenced by both cutaneous and internal thermal receptors. These responses must therefore be described as a function of both skin and core temperature (Brück, 1981; Brück and Zeisberger, 1987). For this, a weighted mean body temperature ($\bar{T}_b$) was calculated according to the following equation:

$$\bar{T}_b = T_{ty} * a + \bar{T}_{sk} * b$$

The coefficients a and b have been found to be 0.87 and 0.13 (Brück *et al.*, 1976; Brück, 1981; Hessemer and Brück, 1985; Hessemer *et al.*, 1986; Brück and Zeisberger, 1987). These weighting factors are comparable with those determined by Nadel *et al.* (1971) for the description of evaporative heat loss. It thus appears to be justified to describe modifications of thermoregulation by an alteration of the threshold temperature for heat production and heat dissipation on the basis of the mean weighted body temperature. The coefficients denote the relative sensitivity of the thermoregulatory responses to the changes of core and skin temperature, but they are not meant to represent weighting factors for the heat content of body core and shell.

Measurement of metabolic rate

Oxygen consumption was measured by an open system consisting of a plastic hood placed over the subject's head, a pump, a flowmeter and a paramagnetic oxygen analyser (Servomex OA 184) as previously described (Hessemer and Brück, 1985). Metabolic rate (MR) was calculated from $\dot{V}_{O_2}$ using a caloric equivalent of 20.4 kJ/l, assuming a respiratory quotient of 0.86.

Measurement of EMG activity

EMG activity was recorded by surface electrodes over the latissimus dorsi and quadriceps femoris muscles. The EMG was integrated and arithmetic means of EMG values from the 2 muscle groups were calculated.

Measurement of sweating rate

Local cutaneous sweating rate (SR) was measured on the chest, using a capsule which covered a skin area of 7 cm². Dried heated air of constant temperature was drawn through the capsule at a constant rate and led to an infrared water vapour analyser (Uras 2t, Hartmann and Braun).

Measurement of local skin blood flow

Local skin blood flow at forearm (λ1) and thenar eminence (λ2) was measured using a heat clearance technique in its six-plate version (Hensel and Brandt, 1977; *see also* Hessemer and Brück, 1985).

Determination of threshold temperatures

Data for metabolic rate, shivering, sweating and skin blood flow were plotted against the mean body temperature. Regression lines for the resting period and the period of activation of each parameter were calculated. The point of intersection of the lines representing the resting and activated periods was called the thermoregulatory threshold temperature.

Hormonal findings

Analysis of the hormones was performed using commercially available radioimmunoassays.

Statistics

For statistical evaluation of the data the Kolmogorov-Smirnov test for the assessment of normal distribution was performed. Since the data were normally distributed, parametric tests (ANOVA, Student's t test) were applied.

Ethics

The protocol of the study was submitted to the local ethics committee and has been fully approved. The informed consent of the patients and control subjects was obtained.

RESULTS

A total of 12 patients and 5 healthy control subjects was investigated. Five patients were examined pre and postoperatively. The anthropometric data are summarized in Table 1. There are no significant differences in body surface area and body surface

TABLE 1 ANTHROPOMETRIC DATA OF PATIENTS AND CONTROLS

		Controls	*Patients*	*P*
Height (cm)	Mean	181.00	168.6	0.005
	SD	7.14	8.4	
	n	5	12	
Weight (kg)	Mean	73.20	76.9	0.390
	SD	12.4	21.4	
	n	5	12	
Body surface area (m^2)	Mean	2.00	1.89	0.204
	SD	0.20	0.26	
	n	5	12	
BSA/BW (m^2/kg)$\times 10^{-4}$	Mean	276.6	256.9	0.112
	SD	21.7	31.4	
	n	5	12	
Age (yrs)	Mean	27.60	42.1	0.006
	SD	1.30	17.0	
	n	5	12	
Sex	M/F	4/1	7/5	

The t test was used for statistical analysis. BSA/BW is the quotient of body surface area and body weight and provides a measure of the body insulation (Baum *et al*, 1976).

area-body weight quotient, the latter being a rough measure of the body heat insulation (Baum *et al.*, 1976). All patients were studied by CT and/or MRI and had established tumours with large suprasellar extensions. An example of a pre and postoperative thermal examination in a patient with a nonsecreting adenoma is given in fig. 1. MR and EMG

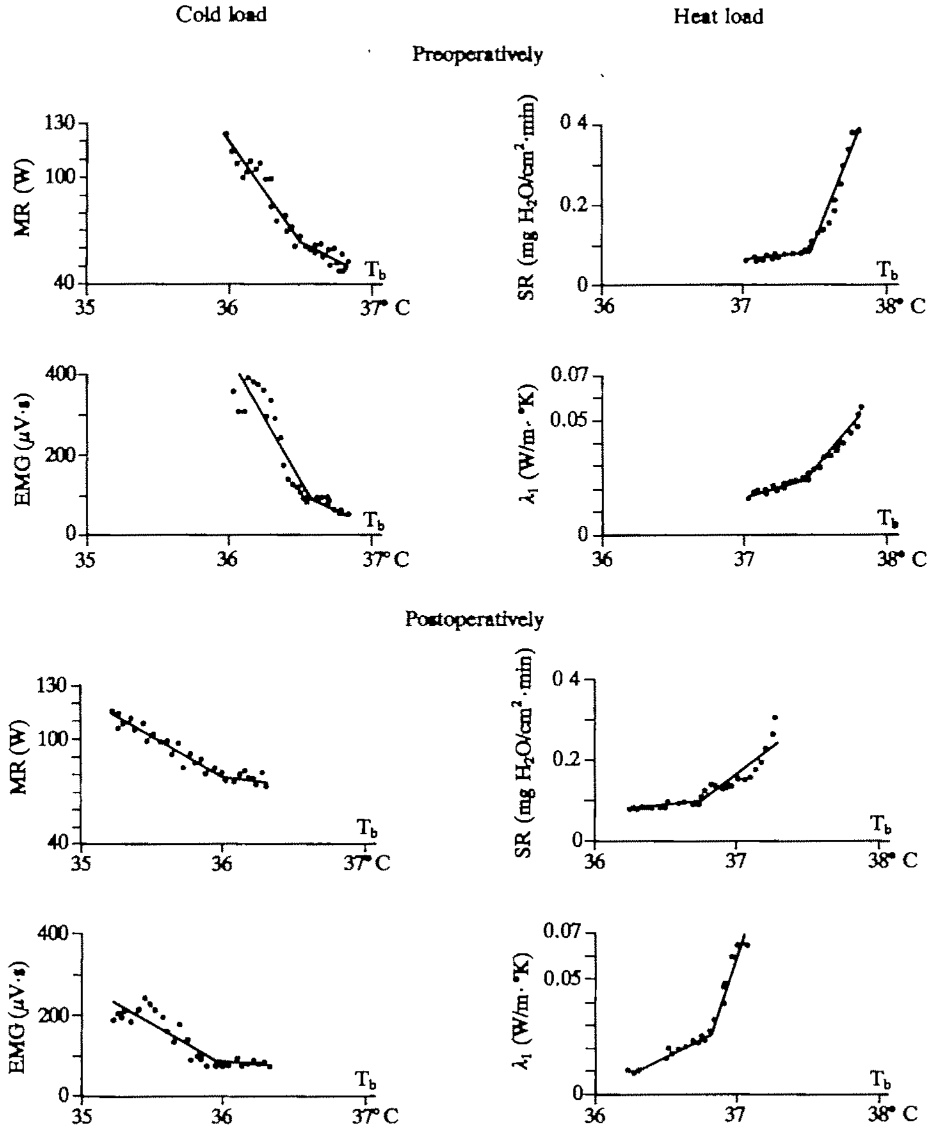

FIG. 1. Thermoregulatory characteristics of a patient with a nonsecreting adenoma (*see* fig. 2). Preoperatively the threshold temperatures for heat production and heat loss are elevated by more than 0.5° C compared with controls. During cold load, MR and EMG activity are increased at 36.6° C $\bar{T}_b$ and heat dissipation by sweating (SR) and vasodilation ($\lambda 1$ = SBF) is activated at 37.5° C $\bar{T}_b$. After operation the threshold temperatures returned to the normal range (bottom left and right). $\bar{T}_b$ = mean weighted body temperature, tympanic temperature used for calculation. MR = metabolic rate; EMG = electromyographic activity; SR = local sweating rate; SBF = $\lambda 1$ = skin blood flow

are plotted against the mean body temperature ($\bar{T}_b$), which is decreasing due to an external cold load (left side). The preoperative threshold temperature was determined at 36.5° C $\bar{T}_b$, which is almost 0.6° C above the normal values reported by Brück *et al.* (1976) and Baum *et al.* (1976). Heat dissipation was assessed preoperatively during heat exposure at 37.5° C $\bar{T}_b$ as outlined in the plot of SR and $\lambda 1$ against $\bar{T}_b$ (upper right side); this value is 0.8° C higher than average values measured in controls. The lower part of fig. 1 illustrates the regression lines and threshold temperatures of the same patient 2 wks after surgery. The threshold temperatures had normalized. Fig. 2 outlines the pre and postoperative radiological findings demonstrating a complete decompression of the hypothalamus after surgery.

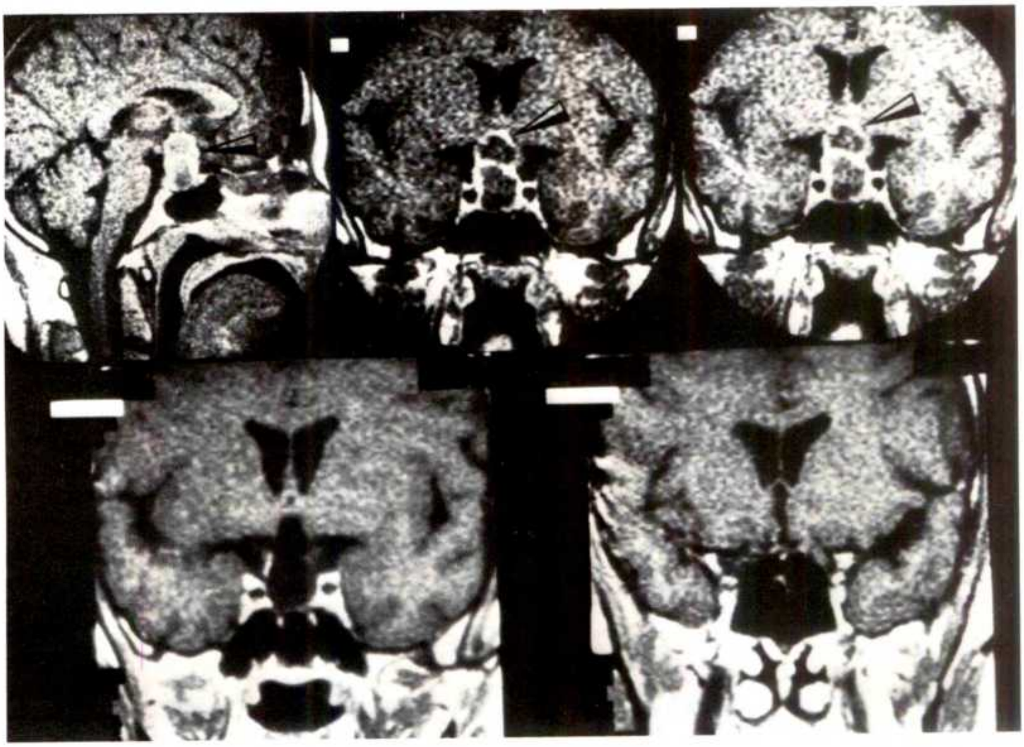

FIG. 2. Magnetic resonance imaging in a patient with a nonsecreting adenoma (*see* fig. 1). Preoperatively the large tumour (arrows) with a suprasellar extension compressed and elevated the floor of the third ventricle (*upper half*). After complete removal (*lower half*) the anatomy appears normal.

The results for 5 patients examined pre and postoperatively are summarized in Table 2. The threshold temperatures for heat gain and heat loss were significantly (ANOVA) elevated in the patients when compared with controls; they returned to the normal range after excision of the tumour. The same result was otained when the thermoregulatory responses were related to core temperature T_{ty}. In 7 other patients who were investigated only preoperatively, similar threshold and body core temperature

TABLE 2 TYMPANIC, SKIN AND BODY TEMPERATURES OF 5 CONTROL SUBJECTS (C) AND 5 PATIENTS PRE AND POSTOPERATIVELY*

	Tympanic temperature				*Mean skin temperature*			*Mean body temperature*			
	C	*Preop.*	*Postop.*	*P*	*C*	*Preop.*	*Postop.*	*C*	*Preop.*	*Postop*	*P*
MR	36.71 (0.091)	37.32 (0.121)	36.72 (0.105)	<0.01	30.93 (0.94)	31.41 (0.611)	30.37 (0.642)	35.96 (0.093)	36.55 (0.039)	35.90 (0.067)	<0.01
EMG	36.69 (0.084)	37.31 (0.119)	36.72 (0.105)	<0.01	31.72 (1.137)	31.64 (0.48)	30.12 (0.638)	36.04 (0.120)	36.58 (0.048)	35.87 (0.068)	<0.01
SR	36.50 (0.109)	37.15 (0.109)	36.54 (0.113)	<0.01	38.25 (0.444)	38.39 (0.349)	38.05 (0.513)	36.73 (0.151)	37.30 (0.094)	36.74 (0.132)	<0.01
SBF	36.44 (0.092)	37.31*	36.50** (0.174)		38.11 (0.414)	38.43*	37.78** (0.314)	36.65 (0.135)	37.44*	36.65** (0.173)	

* Mean values with SEM in parentheses Differences of threshold mean skin temperature are not significant. Patients preoperatively have significantly higher (ANOVA) mean body and tympanic temperatures as compared with postoperative and control values. MR = metabolic rate; EMG = electromyographic activity; SR = sweating rate; SBF = local skin blood flow; * n = 2, ** n = 4

TABLE 3. TYMPANIC TEMPERATURE, MEAN SKIN TEMPERATURE AND MEAN WEIGHTED BODY TEMPERATURE AT SHIVERING THRESHOLD, IN THERMONEUTRALITY AND AT SWEATING THRESHOLD OF PATIENTS PRE AND POSTOPERATIVELY AND OF CONTROL SUBJECTS*

	Threshold-temperatures Shivering			*Temperatures Thermoneutrality*		*Threshold-temperatures Sweating*		
Case	T_{ty}	$\bar{T}_{sk}$	$\bar{T}_b$	T_{ty}	$\bar{T}_{sk}$	T_{ty}	$\bar{T}_{sk}$	$\bar{T}_b$
Preoperatively								
1	37.70	30.40	36.75	37.63	33.97	37.44	38.63	37.55
2	36.95	33.30	36.48	36.95	33.47	36.82	39.11	37.11
3	37.30	32.02	36.61	37.35	32.98	37.25	37.07	37.20
4	37.30	31.14	36.50	37.35	32.51	37.00	38.40	37.16
5	37.34	31.35	36.57	37.31	34.03	37.25	38.74	37.52
6	36.97	31.85	36.30	36.96	34.04	36.86	36.64	36.83
7	37.24	32.35	36.59	37.31	34.65	37.01	35.71	36.85
8	37.26	29.94	36.31	37 15	33.33	37.10	39.87	37.45
9	37.06	31.92	36.38	36 94	32.85	36.95	39.59	37.29
10	36.97	30.36	36.12	37 10	35.52	36.88	36.72	36.86
11	37.12	30.50	36.27	37 20	32.77	36.96	37.94	37.09
12	37 21	30.14	36 83	37.12	34 08	36.97	38.34	37.15
Mean	37.202	31.272	36.476	37 197	33.685	37.041	38.062	37.172
SEM	0.061	0.300	0.060	0 205	0.252	0.054	0.370	0.072
Postoperatively								
1	36.85	28.87	35.82	36.65	34.18	36.66	38.39	36.88
2	36.30	31.87	35 73	36.15	34.49	36.15	38.39	36.45
3	36.83	31.13	36.11	36.90	34.48	36.44	36.33	36.44
4	36.83	28 54	35.78	36.90	34.51	36.79	39.45	37.13
5	36.79	30 20	35.94	36.85	34.40	36.67	37.72	36.81
Mean	36.720	30.122	35 876	36.690	34.412	36.542	38.056	36 742
SEM	0.105	0.638	0 068	0.143	0.060	0.113	0.513	0.132
Control subjects								
1	36 79	32.32	36.20	36.78	34.71	36.75	39.08	37.04
2	36 54	32 15	35 98	36.58	35.38	36.14	36.62	36.20
3	36.45	33.40	36.07	36.45	34.59	36 41	38.28	36 66
4	36.90	27 30	35 64	36.85	34.78	36.54	38.29	36 77
5	36 77	33.44	36 35	36.80	34.29	36.69	39.02	37.00
Mean	36 690	31 722	36 048	36.692	34.750	36.506	38.258	36.734
SEM	0.084	1 137	0.120	0.076	0.178	0.109	0.444	0.151
P	0.0004	n.s.	0 001	0.0004	0.011	0.0004	n.s.	0.009

* 1–5 were examined pre and postoperatively. Significance was tested by ANOVA Significant differences were found between control subjects and patients preoperatively and between patients preoperatively and postoperatively (*see also* Table 2) T_{ty} = tympanic temperature; $\bar{T}_{sk}$ = mean skin temperature; $\bar{T}_b$ = mean weighted body temperature.

displacements as in the other 5 patients (Table 2) were recorded. The data of all 12 patients and 5 controls are collected in Table 3.

In the patients, preoperative shivering ($P = 0.001$, ANOVA) and sweating ($P = 0.006$, ANOVA) thresholds, both in relation to $\bar{T}_b$ as well as the tympanic temperature ($P = 0.001$, ANOVA) were significantly higher than in controls and in the postoperative cases. Significant differences of $\bar{T}_{sk}$ ($P = 0.01$, ANOVA) are only apparent in thermoneutrality, with a lower $\bar{T}_{sk}$ in patients than in controls. At the onset of thermogenesis, however, no difference of $\bar{T}_{sk}$ is detectable.

The resting metabolic rate (RMR) of the control subjects was 51.69 W/m^2 (SEM = 3.66). Because the measurements were obtained in the afternoon, this is slightly above the data of Boothby *et al.* (1936) who found a basal metabolic rate of 48.04 W/m^2 in males and 42.72 W/m^2 in females for the given average body surface area and age. RMR for our patients was 41.38 W/m^2 which was slightly below the standard values of Boothby *et al.* which were 43.74 W/m^2 (males) and 41.82 W/m^2 (females). Even if the older age of our patients and the time of examination (afternoon) are taken into account, their RMR was decreased (Table 4). Comparison of the RMR of the 5 patients examined pre and postoperatively revealed significantly different values of 39.64 W/m^2 preoperatively and 46.63 W/m^2 postoperatively (P = 0.006, t test).

TABLE 4. RESTING METABOLIC RATE [W/m^2] OF PATIENTS AND CONTROLS*

Case	*Patients preop*	*Patients postop.*	*Controls*
1	38.23	50.31	53.76
2	38.57	43.44	58.63
3	40.59	50.24	47.26
4	43.52	47.98	59.03
5	37.32	41.20	39.77
Mean	39.64	46.63	51.69
SEM	1.106	1.845	3.661
6	45.23		
7	40.98		
8	43.02		
9	41.26		
10	40.00		
11	37.42		
12	50.52		
Mean	41.38		
SEM	1.095		

* Difference between patients preoperatively and postoperatively: P = 0.006 (t test); difference between patients preoperatively and control subjects: P = 0.021 (t test).

The preoperative hormonal findings of the patients and the control group of healthy subjects are summarized in Table 5. The basal unstimulated values for thyroid stimulating hormone (TSH), cortisol and adrenocorticotrophic hormone (ACTH) did not differ from controls. The maximal stimulated values were measured in a time period of 0−90 min after the i.v. injection of thyrotropin releasing hormone (TRH, 200 μg), human corticotropin releasing factor (hCRF, 100 μg) and human pancreatic growth hormone releasing factor (GHRF, 100 μg). In the patients there was a moderate increase of TSH and cortisol which was still within the normal range, however. Pilot measurement of T_3, T_4, T_3 uptake and TSH in thermoneutrality and during cold and warm exposure in 5 patients and 3 controls was within the normal range and did not show any change under thermal load. Growth hormone (GH) was subnormal in patients both under basal and stimulated conditions. As 6 patients had prolactin secreting adenomas the average value for this hormone was considerably increased. There was no significant linear or

TABLE 5. BASAL AND MAXIMAL STIMULATED HORMONAL VALUES IN PATIENTS AND CONTROLS*

	TSH		*Cortisol*		*ACTH*		*HGH*		*Prolactin*	
	Basal	*Stim.*	*Basal*	*Stim.*	*Basal*	*Stim.*	*Basal*	*Stim.*	*Basal*	*Stim.*
Controls	1.20	11.26	16.60	29.04	27.25	54.04	1.07	14.47	7.56	48.75
SEM	(0.14)	(0.66)	(0.93)	(1.45)	(4.63)	(6.27)	(0.244)	(1.389)	(0.60)	(4.02)
	n = 30	n = 30	n = 27	n = 27	n = 19	n = 19	n = 23	n = 23	n = 35	n = 35
Patients	1.25	7.62	17.04	21.85	26.44	65.00	0.20	3.99	2944	3912
SEM	(0.26)	(2.02)	(2.31)	(3.60)	(11.15)	(55.00)	(0.035)	(1.817)	(1219)	(1588)
	n = 11	n = 9	n = 11	n = 8	n = 5	n = 2	n = 11	n = 9	n = 11	n = 8
t test	n.s.	$P < 0.05$	n.s.	$P < 0.01$	n.s.	n.s.	$P < 0.01$	$P < 0.01$	$P < 0.01$	n.s.

* Because of the variation of hormonal data even in healthy controls, the control group was expanded by the inclusion of other subjects who were not investigated in the climatic chamber. When comparing thermoregulatory controls alone, there is no significant difference in hormone levels GH = growth hormone; TSH = thyroid stimulating hormone; ACTH = adrenocorticotrophic hormone. Normal ranges: TSH (basal): 0.3–3.3 μU/ml; TSH (stimulated). 5.2–18.6 μU/ml; cortisol (basal): 7.2–26.5 μg/dl; cortisol (stimulated) 14.3–46.4 μg/dl; ACTH (basal) 12–45 pg/ml; ACTH (stimulated). 25–105 pg/ml; prolactin (basal): 3–17 ng/ml; prolactin (stimulated): 14–96 ng/ml; GH (basal): 0.1–4.2 ng/ml; GH (stimulated). 7.2–29.0 ng/ml.

logarithmic correlation between the threshold temperature displacement and increase or decrease of serum prolactin.

The pre and postoperative hormones of the patients whose thermoregulation was investigated pre and postoperatively are given in Table 6. Prolactin was markedly reduced after surgery and stimulation by TRH failed to increase the level of this hormone. The levels of TSH, cortisol and GH did not differ significantly before and after operation.

TABLE 6. HORMONAL FINDINGS IN PATIENTS EXAMINED PREOPERATIVELY AND POSTOPERATIVELY IN THE CLIMATIC CHAMBER*

	TSH		*Cortisol*		*HGH*		*Prolactin*	
	Basal	*Stim.*	*Basal*	*Stim.*	*Basal*	*Stim.*	*Basal*	*Stim.*
Preop.	1.12	9.17	15.32	25.30	0.26	6.55	5219	6604
SEM	(0.49)	(3.94)	(3.89)	(10.46)	(0.07)	(3.84)	(2209)	(2348)
	n = 5	n = 4	n = 5	n = 3	n = 5	n = 4	n = 5	n = 4
Postop.	1.60	9.05	12.17	14.57	0.52	9.07	941	883
SEM	(0.65)	(3.74)	(3.37)	(6.34)	(0.36)	(5.20)	(584)	(496)
	n = 4	n = 4	n = 4	n = 3	n = 4	n = 4	n = 4	n = 4
t test	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	$P = 0.06$	$P = 0.06$

* For control values, normal ranges and abbreviations, *see* footnote to Table 5.

DISCUSSION

The purpose of this study was to investigate the thermoregulatory characteristics of patients with suprasellar pituitary adenomas involving hypothalamic structures. Using thermal load tests, it was possible to detect subtle alterations of the thermoregulatory system which are not detectable by routine temperature measurement in clinical practice. All patients demonstrated an increase of the threshold temperatures for thermoregulatory heat production and heat dissipation, and body core temperature. Their mean skin temperature was significantly lower in thermoneutral ambient conditions. This may be explained by a more pronounced cutaneous vasoconstriction which can be inferred from the vasodilation threshold being displaced to a higher body temperature level (Table 2). By this heat saving measure, it can be assumed that an increased body core temperature (Table 3) can be maintained in spite of a slightly decreased RMR (Table 4). In 5 cases tested after surgery the threshold temperatures, body core temperature, mean skin temperature and RMR were in the normal range.

Threshold displacements to a higher body temperature were reported by Hessemer and Brück (1985) in the luteal phase compared with the follicular phase of young healthy women. Measurement of progesterone was not performed in our study because this mechanism of threshold alteration was not of importance in our patients. The majority of the female patients had menstrual disturbances with absence of the luteal phase due to the adenoma, or were alrady postmenopausal. The others were examined in the follicular phase.

Analysis of basal and releasing factor-stimulated pituitary hormones showed no difference between patients and controls with the exception of GH and prolactin. Stimulation of the other anterior pituitary hormones was insignificantly altered. This excludes any major endocrine impairment, especially of the hypophyseal-thyroid and adrenal axis. Clinical signs of hyper or hypofunction of the adrenals or of hypothyroidism were absent. The latter would be expected to cause hypothermic temperatures; however, an increase in body core temperature was recorded. The role of thyroid function in acute and chronic cold exposure in man is controversial. Fregly (1989) reported several studies demonstrating an increase of TSH during cold exposure, but there is a similar number of publications showing no effect. The reduction of the tympanic temperature by 0.5° C produced by the rapid ingestion of ice did not cause an increase of TSH and GH (Berg *et al.*, 1966). Human newborn infants responded to acute çold exposure with an activation of the pituitary-thyroid axis, but this was not observed in adults (Frohman, 1987). In our patients and control subjects, pilot measurement of thyroid hormones and TSH revealed no change during cold or warm loads.

The level of prolactin did not correlate with the threshold displacement. Half of the patients had tumours without prolactin secretion but displayed a marked threshold elevation.

A major reason for the threshold elevation seemed to be a mechnical effect on hypothalamic structures by compression of the floor of the third ventricle and anterior or medial parts of the hypothalamus. Apart from direct involvement, indirect alterations via disturbances of the local microcirculation, changes in pH and in extracellular and intracellular fluids must be taken into account. It is therefore difficult to correlate the size of the tumour on the basis of radiological investigations with the amount of the threshold shift.

Further support for the hypothesis of the hypothalamic compression as a major reason for threshold deviations is given by 12 patients with midline basal meningiomas, who demonstrated a comparable threshold elevation (Behr *et al.*, 1991). These meningiomas arose from the tuberculum sellae, the sphenoid wing or from the medial part of the sphenoid wing and produced a marked compression of hypothalamic tissue.

In conclusion, this study presents evidence that suprasellar space-occupying lesions in humans influence the central thermoregulatory control system. The observed changes of the regulatory characteristics can be described as a shift of the 'set-point' to a higher temperature level. This 'set-point' shift was reversible after surgery with successful decompression of diencephalic structures.

ACKNOWLEDGEMENT

This study was supported by DFG-Grant Be 1015/1-2.

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(*Received September 6, 1989. Revised April 3, 1990. Accepted April 10, 1990*)

Brain (1991), 114, 709–726

SPINAL CORD SCHISTOSOMIASIS

A CLINICAL, LABORATORY AND RADIOLOGICAL STUDY, WITH A NOTE ON THERAPEUTIC ASPECTS

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SUMMARY

Fourteen patients with schistosomiasis of the spinal cord are described. They fall into two groups: 7 patients had clinical and computer-assisted myelographic evidence of lesions in the conus medullaris or cauda equina, or both. The clinical picture in the remaining 7 patients was usually that of acute or subacute transverse myelitis with normal or equivocal CT myelographic appearances. Granulomas containing bilharzial ova were found in 2 female patients with conus swelling subjected to laminectomy and biopsy. In 1 of these the ova were identified as *S. haematobium* while in the second, unidentified bilharzial ova were found. A presumptive diagnosis of spinal cord schistosomiasis was made in the remaining 12 patients based on characteristic clinical and investigative profiles. All 13 patients tested showed evidence of bilharzial infection based on positive blood serology or the detection of bilharzial ova, or both. All but 1 patient showed at least one of the following abnormalities on CSF examination: pleocytosis, an elevated protein content and a low normal glucose concentration. Unequivocal evidence of intrathecal IgG synthesis was demonstrated as indicated by the presence of oligoclonal IgG bands, an elevated IgG index and an increased CSF IgG synthesis rate. A CSF bilharzia enzyme-linked immunosorbent assay (ELISA) test, developed to indicate the presence of schistosomal infection within the theca, was found to be sensitive although not entirely specific for the diagnosis of spinal cord schistosomiasis.

Eleven of the 14 patients showed rapid clinical improvement, 8 after praziquantel and corticosteroid treatment, 2 after operation and 1 spontaneously. Significant reductions in the CSF cell count, protein concentration and bilharzia ELISA titre, and in intrathecal antibody production, occurred following praziquantel and corticosteroid therapy. The CSF sugar level showed a significant rise. A reduction in conus size was observed in 3 patients when CT myelography was repeated after medical therapy. The normalization of most of the laboratory parameters after combined medical treatment with praziquantel and corticosteroids, together with clinical and radiological improvement, strengthened the presumptive diagnosis of cord schistosomiasis and supports the policy of early intensive medical treatment (as opposed to surgical therapy) for this condition when diagnosed on indirect evidence.

INTRODUCTION

Spinal cord disease is the most common disorder seen in the Neurology Unit at Wentworth Hospital, Durban, accounting for approximately 1 in 4 admissions. The frequent causes of spinal cord disease in our patients, the majority of whom are black (Zulus), include

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trauma, tuberculosis, syphilis, tumours and transverse myelitis; however, the myelopathy remains unexplained in 10% of patients, this constituting the third most common diagnostic category (Wallace and Cosnett, 1983).

In view of the large proportion of cases with unexplained myelopathy, a protocol was established (*see* Appendix) in an attempt to improve the diagnostic yield. This paper examines one possible cause, namely spinal schistosomiasis, and sets out to (1) highlight those clinical features which lead to the suspicion of spinal schistosomiasis; (2) evaluate the role of relatively noninvasive tests (laboratory and radiological) in making a diagnosis of spinal cord schistosomiasis without biopsy; special emphasis was placed on assessing the sensitivity and specificity of CSF markers indicating neurological involvement by schistosomiasis; and (3) study the effect of antischistosomal and corticosteroid therapy on the clinical, laboratory and radiological profiles of patients with a presumptive diagnosis of spinal cord schistosomiasis.

SUBJECTS AND METHODS

With their informed consent, patients with spinal cord disorders were subjected to an investigative protocol (*see* Appendix). With respect to schistosomiasis, investigations were directed as follows.

1. Tests to indicate exposure to the bilharzial parasite: either positive blood bilharzial immunohaemagglution test (BIHA) or the presence of ova (in urine, stool or rectal mucosal biopsy), or both.

2. Appropriate tests to indicate neurological involvement. *Biochemical and serological tests.* (1) CSF microscopy and biochemistry; (2) the presence of bilharzial antibodies in the CSF as detected by an enzyme-linked immunosorbent assay (ELISA) set up by one of us (M.D.P.); and (3) the detection of de novo intrathecal synthesis of immunoglobulin by the IgG index (Lefvert and Link, 1985), the IgG synthesis rate (Tourtellotte *et al.*, 1985) and the demonstration of oligoclonal IgG bands on agarose gel electrophoresis using the method of Mehta *et al.* (1986). The ELISA was performed as described elsewhere (Pammenter *et al.*, 1987) with the following modifications. A soluble egg antigen prepared from *Schistosoma mansoni* (Harn *et al.*, 1984) was used to coat the plates and CSF was diluted 1:10 for the screening assay. To assess the sensitivity and specificity of the CSF bilharzia ELISA test, a control group comprising 87 patients with neurological disorders other than myelopathy were studied. Specifically excluded from this group were patients with an initial diagnosis of meningitis to reduce the influence of a breakdown of the blood-brain barrier. In addition, 140 patients with myelopathy due to causes other than schistosomiasis comprised a second 'control' group. *Radiological examination.* This was undertaken to assess the conus and cauda equina using computer assisted myelography.

Patients thought to have spinal schistosomiasis were treated with praziquantel (PZQ). Nine patients were also given corticosteroids (Table 1). The clinical, laboratory and radiological assessment was repeated after treatment. The values for the CSF cell count, protein and glucose concentrations, and CSF bilharzia ELISA titres before and after treatment of patients with presumed spinal cord schistosomiasis were compared statistically by employing the Wilcoxon matched pairs signed ranks test. The latter nonparametric test was employed since the sample size is small and the data are paired.

RESULTS

Between August 1985 and March 1987 the diagnosis of spinal cord schistosomiasis was made in 14 patients: 12 black (Zulu) and 2 white patients. Two of these patients (Cases 1 and 2 of this study) have been described previously (Cosnett and van Dellen, 1986; Cases 3 and 8, respectively).

Seven patients had clinical and CT myelographic evidence of lesions in the conus medullaris or cauda equina or both (conus group). The clinical picture in the remaining 7 patients was that of acute or subacute transverse myelitis with normal or equivocal

CT myelographic appearances (myelitis group). Two female Zulu patients in the conus group (Cases 1, 6) were subjected to laminectomy. Biopsy in Case 1 revealed granulomas containing *S. haematobium* ova (fig. 1A, B) while in Case 6 unidentified bilharzial ova were found (fig. 1C). However, rectal mucosal biopsy in the latter patient revealed *S. haematobium* ova.

In the remaining 12 cases, a diagnosis of spinal cord schistosomiasis was made without biopsy on the basis of an appropriate clinical and investigative profile (*see below*). As indicated in Table 1 they were treated as follows: (1) PZQ alone, 3 patients; (2) PZQ plus corticosteroids, 9 patients. In the PZQ alone group, 2 patients (Cases 4, 5) received 40 mg/kg/day for 2 days and 1 patient (Case 14) received 50 mg/kg/day for 14 days after he made a spontaneous recovery. In the PZQ plus corticosteroid group all but 1 received 50 mg/kg/day for 14 days. The 1 patient (Case 11) received 25 mg/kg/day for 3 days. The corticosteroid used was usually prednisone at 60 mg/day, generally for 14 days.

Clinical profile

The clinical features are summarized in Tables 1 and 2. Most patients were young, predominantly male and presented with a short history. The duration of symptoms was 1 month or less in 12 patients and approximately 1 yr in the remaining 2 patients (Cases 9, 12). Back pain, lower limb pain and weakness, sensory and sphincter disturbance were present in nearly all patients. Six patients were paraplegic; 1 patient (Case 3) progressed to quadriplegia with respiratory distress.

Clinical outcome

The 2 patients (Cases 1, 6) treated surgically responded well. The clinical details of Case 1 have been documented by Cosnett and van Dellen (1986). Case 6 was an 11-yr-old black female who presented with a flaccid areflexic dense paraplegia, sensory impairment in the lower limbs and urinary retention of 2 wks duration. CT myelography revealed an intramedullary conus lesion. At 6 wks after operation she regained bladder control and walked without support.

One patient (Case 14) a 5-yr-old black male, who presented with flaccid areflexic paraparesis with an L2 sensory level showed spontaneous clinical improvement. Most of the remaining 11 patients showed significant clinical improvement on medical treatment. A striking example was a 16-yr-old black male (Case 3) who presented with a flaccid areflexic quadriplegia, respiratory distress and bisphincter incontinence. Within 6 wks he was bearing weight, walking with assistance and had regained sphincter function; 12 wks after treatment he walked unassisted. Also noteworthy is the rapid clinical improvement observed in 2 patients with conus granulomas (Cases 4, 5) treated with PZQ alone (40 mg/kg for 2 days only). Improvement in either motor or sphincter dysfunction or both occurred from as early as 3 days to 1–2 wks following PZQ with or without corticosteroid therapy, while maximum improvement usually occurred within 4–6 wks of treatment.

Three patients treated medically did not show significant clinical improvement: 1 in the conus group (Case 7) and 2 in the myelitis group (Cases 2, 13). Case 7, initially treated with PZQ alone (40 mg/kg for 2 days only), had persistent urinary retention with overflow incontinence despite a further course of PZQ and corticosteroids. Case 2

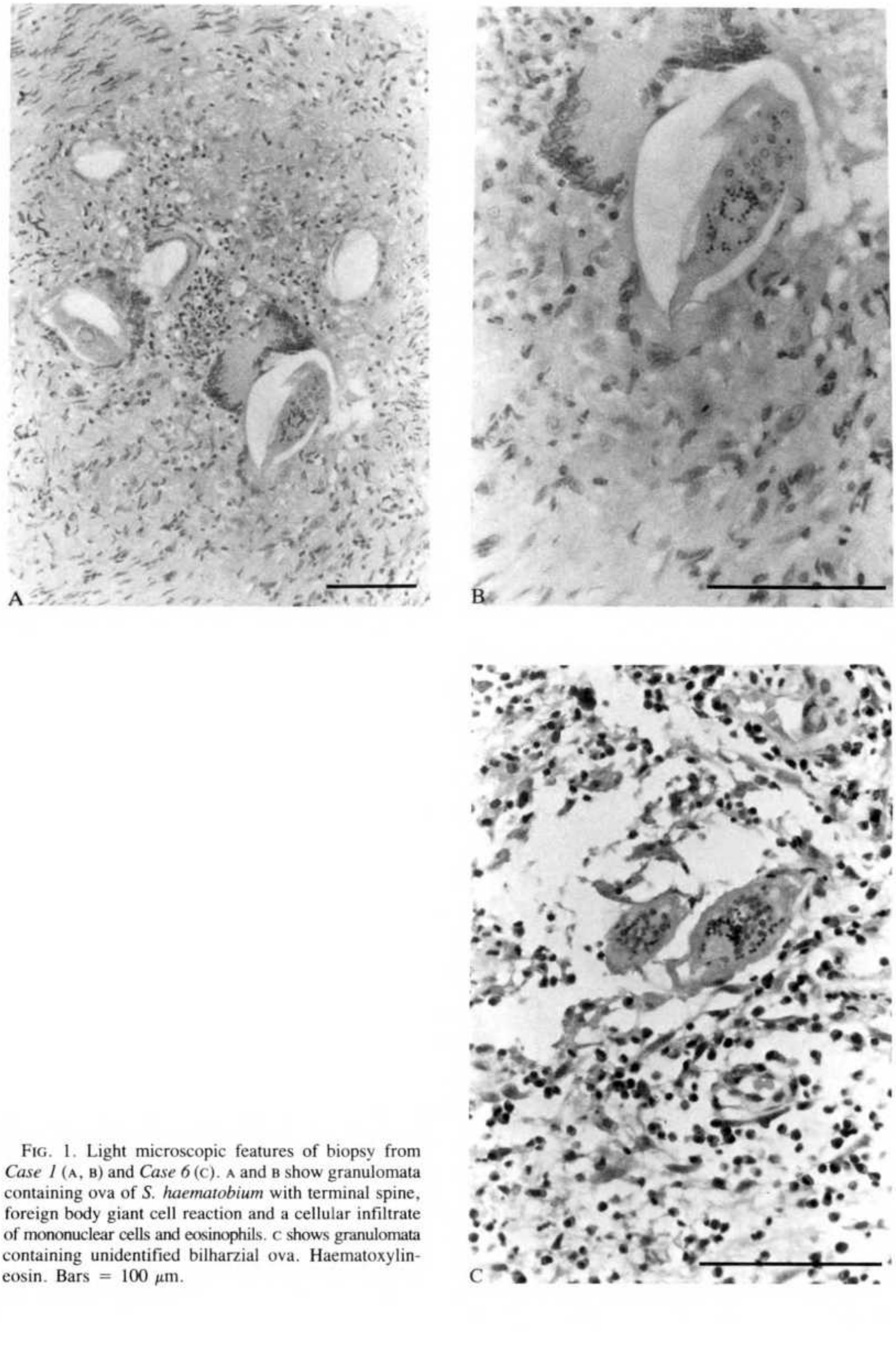


FIG. 1. Light microscopic features of biopsy from *Case 1* (A, B) and *Case 6* (C). A and B show granulomata containing ova of *S. haematobium* with terminal spine, foreign body giant cell reaction and a cellular infiltrate of mononuclear cells and eosinophils. C shows granulomata containing unidentified bilharzial ova. Haematoxylin-eosin. Bars = 100 μm.

TABLE 1 CLINICAL DATA OF 14 PATIENTS WITH SPINAL CORD SCHISTOSOMIASIS

Case	*Age (yrs)*	*Race*	*Sex*	*Clinical presentation*	*Treatment*	*Outcome*
1	6	B	F	CG	Operation	Good
2	65	W	M	MY	PZQ+CST	Poor
3	16	B	M	MY	PZQ+CST	Good
4	37	B	F	CEA	PZQ	Good
5	13	B	M	CG	PZQ	Good
6	11	B	F	CG	Operation	Good
7	13	B	M	CG	PZQ → PZQ+CST	Poor
8	19	W	M	MY	PZQ+CST	Good
9	11	B	M	CG	PZQ+CST	Good
10	41	B	M	MY	PZQ+CST	Good → relapse
11	35	B	M	MY	CST → PZQ+CST	Good
12	30	B	M	MY	PZQ+CST	Good
13	8	B	M	MY	PZQ+CST	Poor
14	6	B	M	CG	PZQ	Good

M = male; F = female; B = black; W = white; CG = conus granuloma with cauda equina arachnoiditis; CEA = cauda equina arachnoiditis with possible conus granuloma; MY = myelitis; PZQ = praziquantel; CST = corticosteroids.

TABLE 2 SYMPTOMS AND SIGNS AT PRESENTATION IN 14 PATIENTS WITH SPINAL CORD SCHISTOSOMIASIS

	Conus	*Myelitis*	*Total*	*%*
Back or lower limb pain or both	6	5	11	79
Lower limb weakness	7	7	14	100
Asymmetric	5	3		
Symmetric	2	4		
Unable to walk	5	5		
Able to walk	2	2		
Tone changes	6	7	13	92
Hypotonia	5	5		
Hypertonia	1	2		
Reflex changes	7	7	14	100
Absent	5	4		
Brisk	1	3		
Asymmetric	1			
Sensory dysfunction	7	7	14	100
Sensory level				
Sacral	2			
Lumbar	4	3		
Lower thoracic	1 (T12)	2 (T10)		
Midthoracic		1 (T6)		
Cervical		1		
Sphincter dysfunction	5	7	12	86

showed cord atrophy (fig. 2) at follow-up CT myelographic study performed 4 months after initial presentation. He remained wheelchair-bound with sphincter dysfunction at follow up 2½ yrs after initial presentation. Case 13 was followed up for 2 wks only following medical treatment, at which stage flaccid paraplegia and bisphincter dysfunction remained. It is noteworthy that both patients with a necrotic transverse myelitis showed *S. mansoni* on rectal mucosal biopsy. One patient (Case 10) showed marked clinical improvement after a 2 wk course of PZQ and corticosteroids. However, after 14 months

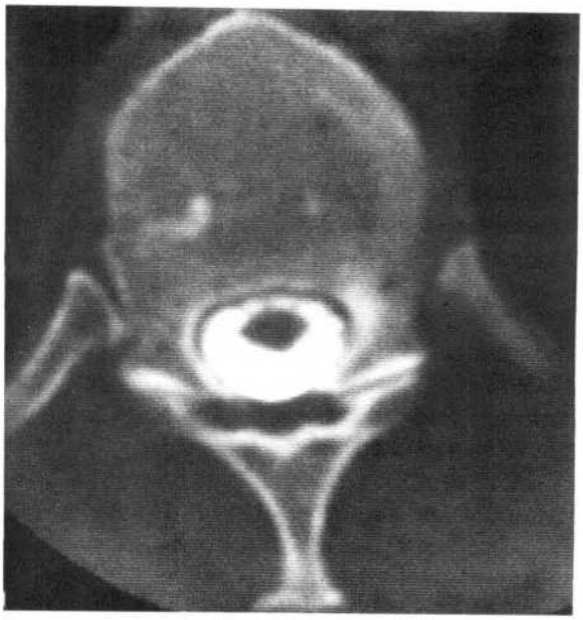


FIG. 2. Computer-assisted myelographic study of *Case 2* showing cord atrophy at lower thoracic level, 4 months after initial presentation with necrotic transverse myelitis. The cord cross-sectional area is only about one-fifth of that of the spinal canal.

the patient presented again with paraplegia of 3 months duration. He was treated with a further course of PZQ and corticosteroids but did not respond on this occasion.

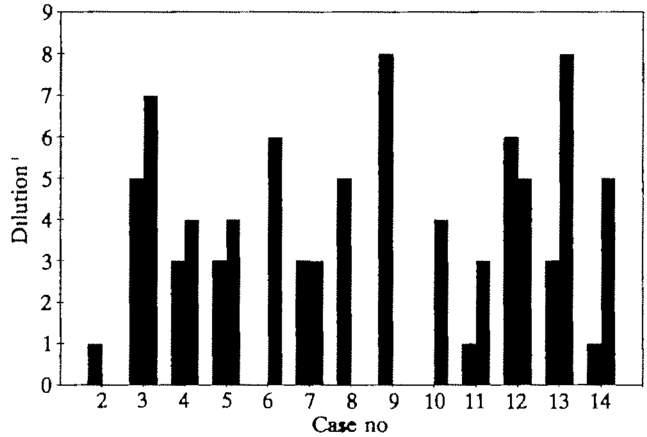
Investigations

Absolute eosinophil count. This ranged from 78 to 9729 cells/mm^3 with a median of 880/mm^3. Eosinophilia, defined as an eosinophil count above 700/mm^3, was present in 7 of the 13 patients in whom the eosinophils were counted.

Bilharzial ova. These were found in 10 of 12 patients in whom rectal biopsy was undertaken. Of these *S. haematobium* infection was noted in 4 patients, *S. mansoni* infection in 3 patients and unidentified schistosomal infection in 3 patients. In 2 further patients *S. haematobium* ova were identified in the urine. In 1 of the 3 patients in whom *S. mansoni* ova were identified on rectal mucosal biopsy, *S. haematobium* ova were also detected on stool examination.

Antibilharzial antibodies. These were detected in the blood in all 13 patients in whom the test was performed. Fig. 3 shows that the bilharzia IHA titre varied from 1:16 to more than 1:2048. Titres rose in 6 patients following specific treatment.

CSF profile (Table 3). CSF was not available in every case for studies before and after treatment. The interval between CSF measurements made before and after treatment varied from 3 wks to almost 1 yr with most patients having repeat CSF study within 12 wks. Fig. 4 illustrates the CSF profile before and after treatment in patients in whom a presumptive diagnosis of spinal schistosomiasis was made. A significant reduction in the CSF cell count ($P = 0.005$; n = 11) and protein level ($P = 0.021$; n = 10) and a significant rise in the CSF glucose level ($P = 0.021$; n = 8) resulted following medical therapy.



*1=1.16:3=1 64,4=1:128,5=1·256;6=1 512,7=1.1024;8=1 2048

FIG 3. Bar graph showing bilharzia IHA titre before and after treatment. Hatched bars indicate titre before treatment and solid bars the titre after treatment

TABLE 3 CSF PROFILE IN 14 PATIENTS WITH SPINAL CORD SCHISTOSOMIASIS BEFORE AND AFTER TREATMENT

	CSF cell count (No./mm³)		*CSF protein (g/l)*		*CSF glucose (mmol/l)*		*CSF bilharzia ELISA units*	
Case	*Before*	*After*	*Before*	*After*	*Before*	*After*	*Before*	*After*
1	82	ND	1.0	ND	2.2	ND	ND	ND
2	60	0	1.8	0.99	2.8	3 7	ND	124.3
3	38	0	0.8	0.4	3.1	ND	41.2	8.1
4	56	ND	ND	ND	3.2	ND	44.7	ND
5	12	6	1.52	ND	2 7	2.6	ND	ND
6	22	4	0.92	0.2	2 5	3 1	ND	28.2
7	28	0	0 5	0.26	2.8	3.3	ND	49 6
8	32	4	1.2	1.4	2.9	ND	148 7	93.4
9	18	2	0.56	0 26	2 5	2.8	193 9	46.1
10	10	2	0.52	0.54	3.4	3.8	53.2	21.7
11	30	2	0.9	0.4	2.8	3.1	42 1	14.2
12	0	0	0.32	0 32	3 1	ND	62.9	47.7
13	16	6	0 4	0.16	2 3	2.9	101.1	75 0
14	6	0	1.08	0.48	2.8	2 9	168 9	118.9

ND = not done

The ELISA for specific antischistosomal antibodies was performed on 9 patients before treatment, 7 of whom had values greater than 3 SDs of the control group (43 units). The remaining 2 patients (Cases 3, 11) had values of 41.2 and 42.1 units, respectively. A further 3 patients were tested only after treatment; 2 of these (Cases 2, 7) also had raised antibody levels. The third patient (Case 6) was tested almost 1 yr after operation. Thus, of the 12 patients tested before or after treatment, 9 (75%) had ELISA values greater than 3 SDs of the control group, while of 140 patients with myelopathy due to other causes (including tuberculosis, syphilis, HTLV-I associated myelopathy (HAM),

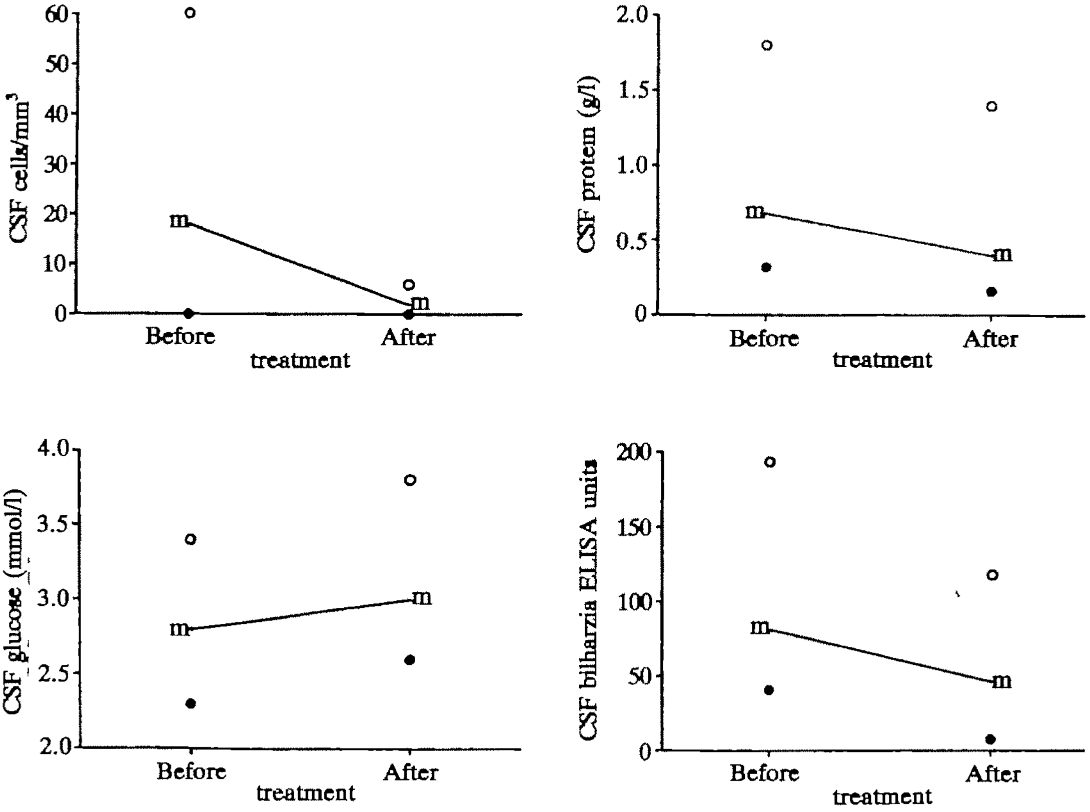


FIG. 4. Median ('m'), maximum (open circle) and minimum (closed circle) CSF values for patients with presumptive diagnosis of spinal schistosomiasis before and after treatment. In each CSF perameter the change was significant ($P < 0{\cdot}05$). *See* text for details.

myelitis, tumours, spondylosis, cysticercosis, arachnoiditis, syringomyelia, idiopathic) only 10 (7%) had such values. The CSF bilharzia ELISA titres also fell significantly ($P = 0.012$; $n = 8$) after treatment (fig. 4). Oligoclonal bands, present in the CSF but not in the serum, were clearly demonstrable in 5 of 9 cases tested (fig. 5). The other 4 had a single band in the CSF absent in the serum but this could not be proven to be IgG by immunoprecipitation.

Synthesis of IgG within the blood-brain barrier was assessed in 3 patients (Cases 9, 10, 11) before and 11 wks, 5 wks and 8 wks, respectively, following medical therapy (PZQ and corticosteroids). As seen in Table 4, unequivocal evidence of de novo CNS IgG synthesis was demonstrated; furthermore, medical treatment consistently reduced the CSF IgG content and de novo intrathecal IgG production.

Recently, HTLV-I infection has been associated with 'unexplained myelopathy' in tropical and subtropical countries (Gessain *et al.*, 1985; Osame *et al.*, 1986). Antibodies to HTLV-I in the CSF were not detected in the 12 cases tested.

Radiology. CT myelography showed conus expansion with irregularity and matting of roots in 6 patients (figs 6A, B, C, 7).

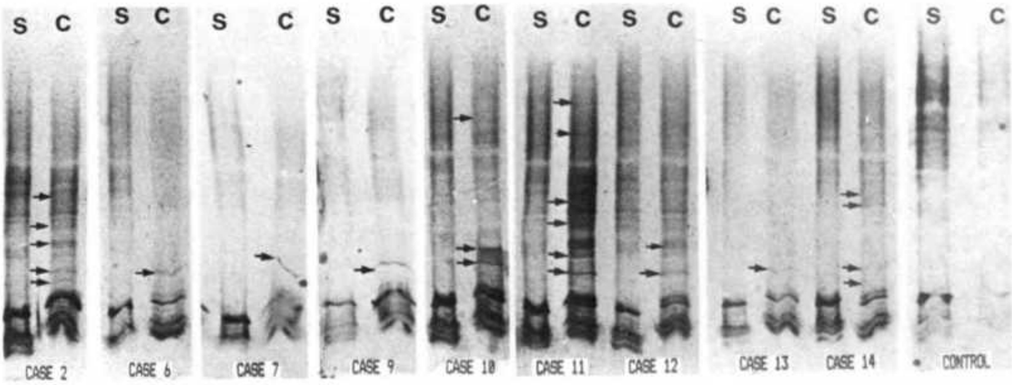


FIG. 5. Serum and CSF separated by isoelectric focusing using Resolve ampholytes (Isolab Inc., Ohio, USA). A gradient of pH 3 – 10 was formed using 2 parts 3 – 10 ampholytes and 1 part 7 – 9 ampholytes. Equal amounts of serum and CSF IgG were loaded in each case and the gel stained with silver. Arrows show bands present in the CSF but not in the serum. *See* text for details.

TABLE 4. INTRATHECAL IgG SYNTHESIS IN 3 PATIENTS WITH SPINAL CORD SCHISTOSOMIASIS BEFORE AND AFTER TREATMENT WITH PRAZIQUANTEL AND CORTICOSTEROIDS

	Case 9		*Case 10*		*Case 11*	
	Before	*After*	*Before*	*After*	*Before*	*After*
Serum albumin (g/l)	40.9	37.6	46.5	40.6	41.9	40.5
Serum IgG (g/l)	13.73	11.42	13.49	9.68	16.55	15.10
CSF albumin (g/l)	0.47	0.19	0.29	0.31	0.88	0.44
CSF IgG (g/l)	0.45	0.046	0.077	0.043	0.040	0.010
IgG Index*	2.86	0.8	0.92	0.58	1.15	0.6
IgG synthesis (mg/day)**	185.8	5.8	12.1	1.5	118.3	8.4

* The normal IgG index is less than 0.7. ** The normal de novo CNS IgG synthesis rate is less than 3.3 mg/day.

One patient (Case 4) showed possible conus enlargement but definite cauda equina arachnoiditis. The myelographic appearances in the remaining 7 patients who presented as acute or subacute transverse myelitis were normal or equivocal. CT myelography was repeated in 3 patients with conus expansion (Cases 5, 7, 9) 3 wks, 4 months and 9 wks, respectively, after medical treatment. There was a decrease in conus size in all 3 cases. Fig. 8 shows comparative reformatted images of the conus in Case 9 when CT myelography was done before and approximately 9 wks after combined treatment. Midline sagittal images (fig. 8A) showed a decrease in the anteroposterior diameter of the conus by 2.1 mm from 11.3 mm before treatment to 9.2 mm after treatment. The coronal images (fig. 8B) showed a decrease in the transverse diameter of the conus by 1.2 mm from 10.8 mm before treatment to 9.6 mm after treatment. The radiological improvement mirrored the improvement noted clinically and in the CSF in 2 of the 3 patients (Cases 5, 9). In the third patient (Case 7), despite a significant reduction in conus size noted in CT myelography 1 yr after treatment, urinary retention with overflow incontinence remained a problem. CT myelography, however, did show extensive arachnoiditis.

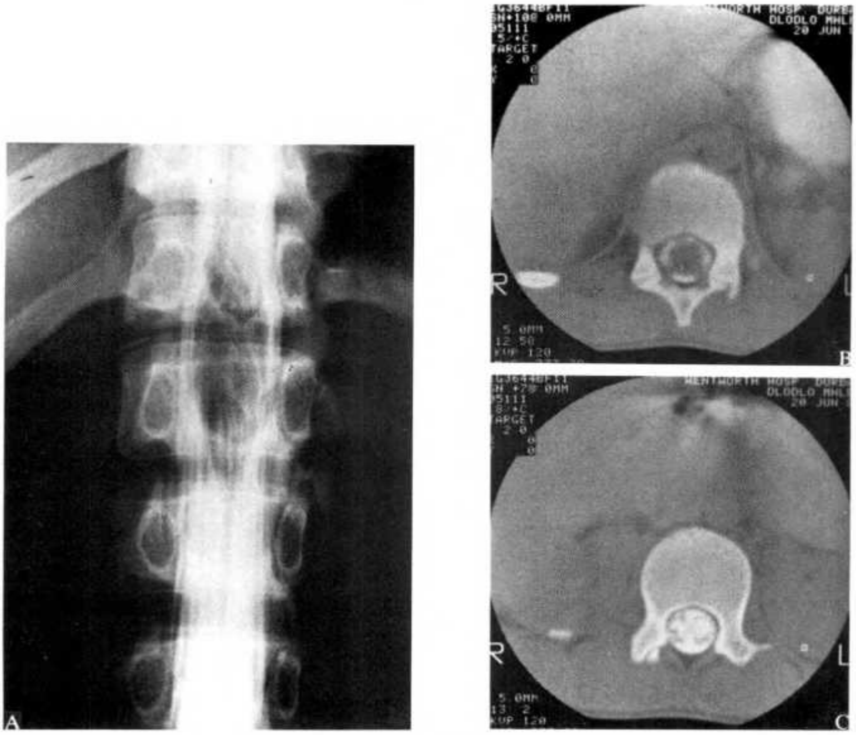


FIG. 6. Computer-assisted myelographic study in *Case 6.* A, myelogram showing expansion of conus medullaris maximum opposite T11/T12 vertebrae. B and C, CT myelograms showing conus enlargement (B) and irregularity and matting of nerve roots of cauda equina (C).

DISCUSSION

Prevalence

The reported prevalence of spinal schistosomiasis is low. A recent literature search yielded only 39 cases of schistosomiasis of the spinal cord reported in the French and English language since January 1969 (Cosnett and van Dellen, 1986). It is our belief that spinal schistosomiasis is an under-reported condition. For example, in 1985, before the myelopathy protocol was fully adopted, we diagnosed spinal schistosomiasis in only 1 of 106 patients (0.9%) with spinal cord disorders. In 1986, however, 12 of 179 (6.7%) patients with spinal cord disease were shown to have spinal schistosomiasis.

Worldwide, *S. mansoni* is the more frequently reported cause of schistosomal myelopathy while in Africa *S. haematobium* is more often responsible (Scrimgeour and Gajdusek, 1985). This has also been our experience. *S. haematobium* ova were found on biopsy in 1 case in whom surgery was performed. This case was a young Zulu female. Histologically proven cases of schistosomal myelopathy are rare in women (Scrimgeour, 1984). There seems to have been only 1 other female patient with *S. haematobium* myelopathy previously reported (Gelfand, 1950). Case 1 therefore probably represents the second histologically proven case of *S. haematobium* myelopathy documented in

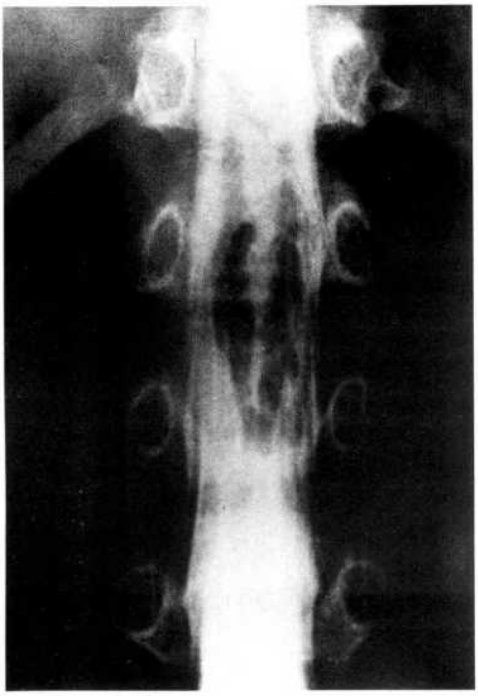


FIG. 7. Myelogram in *Case 9* showing conus expansion with irregularity of nerve roots of cauda equina.

a female. In the remaining 13 patients *S. haematobium* infection was noted in 6 patients, *S. mansoni* infection in 2 patients, *S. haematobium* and *S. mansoni* in 1 patient, and unidentified schistosomal infection in 3 patients; in 1 patient schistosomal infection was presumed from clinical and biochemical characteristics.

Clinical presentation

The clinical presentation may reflect one of two main pathological processes: (1) conus granuloma or cauda equina arachnoiditis, or both; (2) necrotic myelitis (Case Records of the Massachusetts General Hospital, 1985; Cosnett and van Dellen, 1986). The symptoms and signs of the former process are usually those of a conus or cauda equina syndrome and the sensory level is hence very rarely above T12. Only 1 of our 7 patients in the conus granuloma group had a T12 sensory level. Urinary retention is occasionally the presenting symptom, as occurred in 1 of our patients. The highest reported sensory level in the granuloma group is at C8 in a quadriplegic patient in whom a large mass demonstrated radiologically in the cervical spinal canal almost completely disappeared after treatment with niridazole (Molyneux and Galatius-Jensen, 1978).

The clinical picture in the myelitis group is usually that of an acute or subacute transverse myelitis. It differs from other causes of transverse myelitis in that schistosomal transverse myelitis commonly affects the lower cord whereas other causes of transverse myelitis commonly affect the midthoracic region (Tyler, 1982). In this study, 5 of the 7 patients with a myelitic presentation had a sensory level at or below T10. However, 1 patient had a T6 sensory level while the highest sensory level in this study was low cervical in a quadriplegic patient who presented with proprioceptive loss in the fingers.

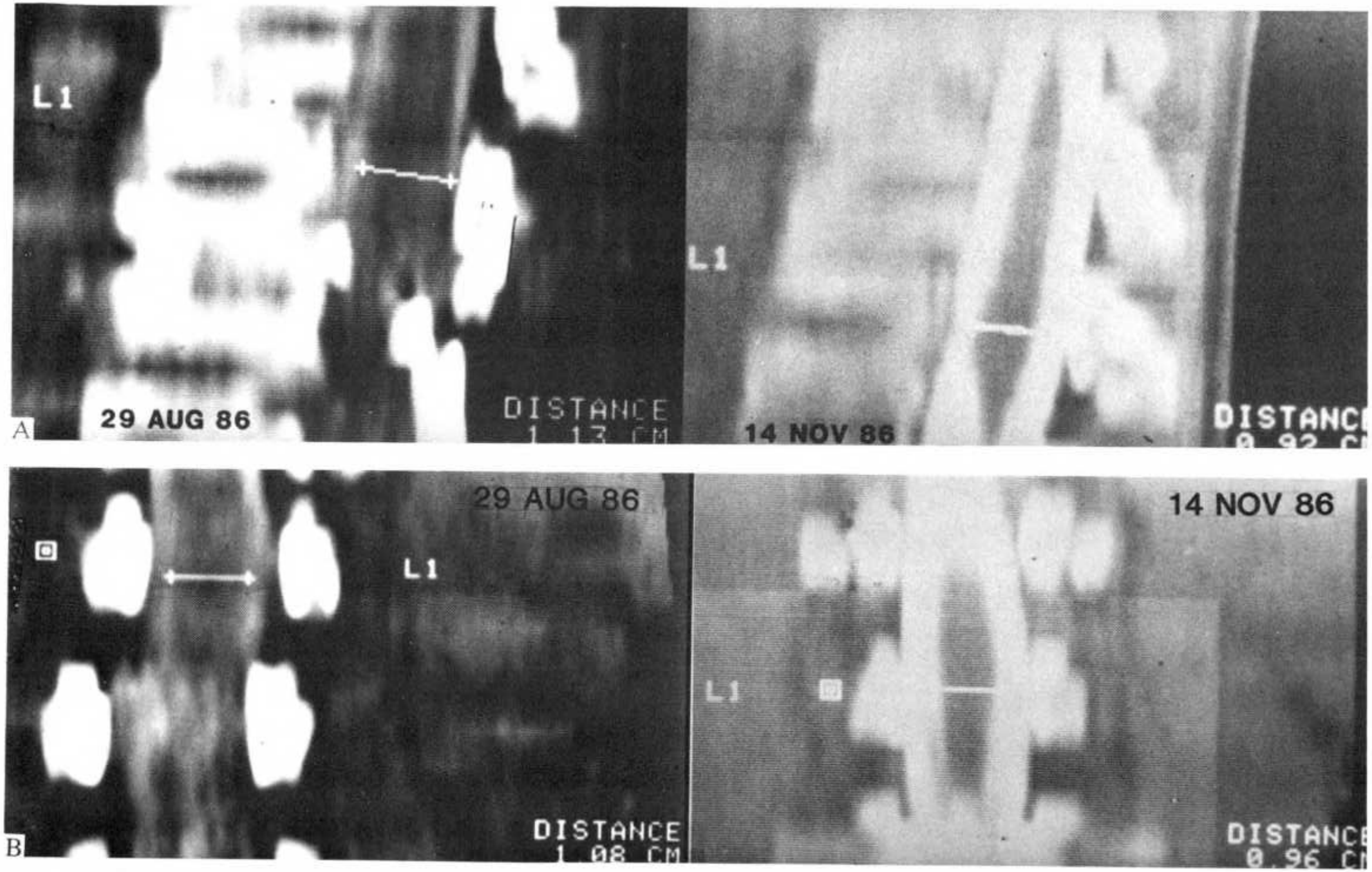


FIG. 8. Comparative reformatted images of the conus in *Case 9* when computer assisted myelography was performed before (29.8.86) and approximately 9 wks after completion of a 2 wk course of PZQ and corticosteroids (14.11.86). A, midline sagittal images showing a decrease in the anteroposterior cord diameter by 2.1 mm from 11.3 mm before treatment to 9.2 mm after treatment. B, coronal images showing a decrease in the transverse cord diameter by 1.2 mm from 10.8 mm before treatment to 9.6 mm after treatment.

This is the highest level recorded in schistosomal transverse myelitis. Previously an autopsy study has shown total myelonecrosis below T4 with demonstration of ova of *S. mansoni* in the necrotic cord tissue and leptomeninges (Queiroz *et al.*, 1979).

Investigations

When autopsy or surgical biopsy is not performed, a presumptive diagnosis of spinal cord schistosomiasis is based on (1) evidence of lower thoracic/upper lumbar neurological lesions; (2) the demonstration of exposure to schistosomiasis through parasitological or serological techniques; and (3) the exclusion of other causes of transverse myelitis (Houpis *et al.*, 1984).

The diagnosis of conus granuloma is easier in view of its delineation on computer assisted myelography. The diagnosis of schistosomal myelitis, however, is circumstantial and is based on the three conditions mentioned above. Exposure to schistosomiasis through parasitological or serological techniques was demonstrated in all 13 patients in whom it was sought. However, in an endemic area, demonstration of visceral schistosomiasis merely reflects exposure to the bilharzial parasite. Hence, in this environment, where cord dysfunction of unknown cause is frequent, reliance on the above three conditions could lead to a wrong diagnosis of spinal schistosomiasis. The second part of our study, therefore, was directed towards demonstrating intrathecal schistosomal infection with the aid of laboratory and radiological tests.

Laboratory tests. Mild CSF pleocytosis (up to 100 cells/mm^3) as shown in 13 of the 14 patients, was the rule in spinal schistosomiasis. Of greater diagnostic relevance was, first, the demonstration of raised levels of specific antischistosomal antibodies in the CSF in 9 of the 12 cases tested (75%). This contrasts with only 7% ($n = 140$) of other myelopathy cases in whom raised antibody levels were found. Secondly, intrathecal production of antibodies was demonstrated in our patients as indicated by an elevated IgG index, increased IgG production rates and the presence of oligoclonal bands in the CSF. This has not previously been reported in spinal schistosomiasis.

Recently, HTLV-I infection has been associated with unexplained myelopathies in tropical and subtropical countries (Gessain *et al.*, 1985; Osame *et al.*, 1986). None of the patients with presumed spinal schistosomiasis was positive for HTLV-I. It is important to exclude HTLV-I infection in this series of patients with a presumptive diagnosis of cord schistosomiasis for several reasons. First, the geographic distribution of HAM is somewhat similar to that of schistosomiasis (Mahmoud, 1984). Secondly, the clinical picture may be similar when patients present with spastic paraparesis. Thirdly, CSF pleocytosis and elevated protein concentrations occur in both conditions (Osame *et al.*, 1987; Rodgers-Johnson *et al.*, 1988; Bhigjee *et al.*, 1990). Fourthly, corticosteroids are of therapeutic benefit in spinal schistosomiasis and sometimes in HAM (Osame *et al.*, 1987). However, an important difference is that most patients with spinal schistosomiasis are male and younger whereas those with HAM are female and older (Rodgers-Johnson *et al.*, 1988; Bhigjee *et al.*, 1990).

Radiological examination. The characteristic myelographic features in schistosomal myelopathy have been well described by El-Banhawy (1971). Where the pathology of cord schistosomiasis is a granuloma, computer assisted myelography demonstrates irregular swelling of the lower spinal cord or conus medullaris. A normal myelogram does not exclude the diagnosis as a CT scan may demonstrate spinal cord swelling in such cases (Houpis *et al.*, 1984). In most cases irregular swelling of the nerve roots forming the cauda equina is frequently associated with conus swelling (Kerr *et al.*, 1987). Cauda equina arachnoiditis may, however, be the predominant feature as was seen in 1 patient.

The radiographic appearances on computer assisted myelography in patients with myelitis are usually normal. Cord atrophy may be demonstrated later in the disease in patients with necrotic myelitis. This explains the poor prognosis in such patients.

Therapeutic aspects

Further evidence to support the presumptive diagnosis of spinal schistosomiasis was the favourable response to treatment with PZQ and corticosteroids on the clinical, laboratory and radiological profiles of these patients.

A consistent feature is the rapid onset of clinical improvement, occurring usually within a few days, following the commencement of PZQ used alone or in combination with corticosteroids. Remarkable improvement in motor, sensory and sphincter function occurred in most patients, usually by 6 wks. One patient showed spontaneous clinical improvement. Such spontaneous remission for prolonged periods has been commented upon by other authors (Raper, 1948; Lechtenberg and Vaida, 1977). The poor response observed in 3 patients may be due to irreversible structural cord damage which, in turn, was probably related to delay in diagnosis and treatment. The latter explanation also accounts for the poor outcome in the 1 patient who relapsed 14 months after an excellent initial response to PZQ and corticosteroids. The value of early antischistosomal and corticosteroid therapy has been emphasized by others (Bird, 1978; Cosnett and van Dellen, 1986).

Parallel with clinical improvement is the improvement observed in the CSF pleocytosis and chemistry after treatment. Furthermore, there was a decrease in the specific antischistosomal antibody titre and the intrathecal IgG synthesis rate. This change in the rate of de novo CNS IgG synthesis can be used to follow the effect of putative therapies (Tourtellotte and Ma, 1978). The trend to normality of these CSF parameters after treatment lends support to the premise that our diagnosis of spinal schistosomiasis is correct. Measurements of cord diameter before and after medical treatment are of value because a significant reduction in cord swelling many weeks to months after treatment also helps to strengthen a diagnosis of cord schistosomiasis. However, transient improvement has been observed after some cord tumours have been treated with oedema-reducing steroids. Treatment regimes using PZQ and corticosteroids varied in dosage and duration. While successful treatment has been shown with oxamniquine (Efthimiou and Denning, 1984), niridazole (Bird, 1978; Molyneux and Galatius-Jensen, 1978) and combination drug treatment (Kerr *et al.*, 1987), we believe that we have shown for the first time that PZQ alone can also be effective in cord schistosomiasis, as occurred in 2 of our patients.

The mode of action of antischistosomal agents in spinal schistosomiasis is unknown. One suggestion is that these drugs act by killing the adult female worms and, as ova production ceases, inflammatory responses subside (Scrimgeour and Gajdusek, 1985). However, this explanation seems unlikely to account for the rapid response (measured in days) to treatment with PZQ in cord schistosomiasis. While PZQ has been reported not to be ovicidal (Andrews *et al.*, 1983), this view is not universal (Xu *et al.*, 1988).

Whether PZQ has immunosuppressive or anti-inflammatory actions is controversial. Madwar *et al.* (1983) believe that PZQ reduces immunological reactions but Tawfik and Colley (1986) point out that there are no convincing data to support such a conclusion. More recently it has been shown in a mouse model that antischistosomal antibody levels fall following praziquantel administration (Xu *et al.*, 1988).

The role of corticosteroid is also controversial in spinal schistosomiasis (Scrimgeour and Gajdusek, 1985) but we believe its therapeutic efficacy should not be underestimated. By suppressing the inflammatory response, granuloma formation and tissue destruction is halted (Domingo *et al.*, 1967; Claman, 1972).

Case 11, who had a dense paraplegia, demonstrated the value of corticosteroids in spinal schistosomiasis. He was initially thought to have 'idiopathic' transverse myelitis and given corticosteroids empirically. Within 3 days improvement, to the extent that he attempted to bear weight, was observed. PZQ was started only 5 days later when the urine showed viable *S. haematobium* ova. Improvement with corticosteroid therapy has also been mentioned by others (Wakefield *et al.*, 1962; Bird, 1964; Herskowitz, 1972; Rosenbaum *et al.*, 1972; Saxe and Gordon, 1975; Lechtenberg and Vaida, 1977). Thus although there have been no controlled studies, the use of corticosteroids as soon as the diagnosis of spinal schistosomiasis is suspected is probably justified. Moreover, to undertake controlled clinical trials would be impossible due to the relative rarity and the variability of pathology and clinical presentation of the condition (Cosnett and Haribhai, 1990).

Although there has been no long-term follow-up in our series, no cases with presumed spinal schistosomiasis treated with this regime have yet been shown to have a different diagnosis. The significant reduction in conus size found on CT myelography, up to 1 yr after PZQ and corticosteroids have been discontinued, is reassuring evidence that, in employing this regime, the presence of other neoplasms or cord granulomas is not masked.

CONCLUSIONS

Spinal cord schistosomiasis has a characteristic clinical and investigative profile. This allows such a diagnosis to be made confidently using relatively noninvasive tests without resorting to laminectomy and biopsy. Further support for the diagnosis of spinal cord schistosomiasis is the rapid and marked improvement observed in the clinical, laboratory and radiological profiles following antischistosomal drugs used alone or in combination with corticosteroids. Treatment must be instituted early to reduce morbidity due to irreversible neurological damage. These points are especially relevant in the management of patients presenting in rural areas of under-developed tropical and subtropical countries where sophisticated investigative facilities are not readily available. When spinal schistosomiasis is suspected the rural doctor is advised to undertake a therapeutic trial with PZQ and corticosteroids promptly, even without direct proof of diagnosis (Cosnett and Haribhai, 1990). The patient should be referred to a neurological centre for more detailed evaluation while treatment is in progress.

We believe that this is the first relatively large comprehensive prospective study examining the clinical, laboratory, radiological and therapeutic aspects of schistosomiasis affecting the spinal cord. The CSF bilharzia ELISA test developed to indicate the presence of schistosomal infection within the theca is found to be sensitive, though not entirely specific, in the diagnosis of cord schistosomiasis. The normalization of most of the laboratory abnormalities after medical treatment, including a fall in CSF cell count, protein level, CSF bilharzia ELISA titre and intrathecal antibody production, together with clinical and radiological improvement, not only indicates control of the infection but also offers confirmation of the diagnosis when this has been in question (Cosnett and Haribhai, 1990). No patient diagnosed to have spinal schistosomiasis on indirect evidence deteriorated through misdiagnosis. On the contrary, prompt appropriate therapy based on presumptive diagnosis probably prevented lasting physical disability.

ACKNOWLEDGEMENTS

The expert technical assistance of Mrs S. R. Epstein and Mrs E. J. Rossouw is gratefully acknowledged. We are indebted to Dr J. E. Cosnett for his critical reading of the manuscript and Dr R. M. Cooppan for advice and comments. The secretarial assistance of Miss P. Enstrom, Mrs C. McKeown and Mrs K. George is most appreciated. We are indebted to Dr K. Cooper, Dr G. Haripersadh and Dr A. Bramdev for examination of the histological material and preparation of the photomicrographs. The painstaking effort of the Medical Illustration Unit, University of Natal, is gratefully acknowledged.

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(*Received September 19, 1989. Revised March 19, 1990. Accepted April 11, 1990*)

APPENDIX: MYELOPATHY PROTOCOL

Blood tests

Full blood count.
Erythrocyte sedimentation rate.
Liver function tests
Serum protein electrophoresis.
Serum immunoglobulins.
Antinuclear factor.
Serum angiotensin converting enzyme.
Serum folate and vitamin B12
Serological tests for syphilis.
Bilharzia immunohaemagglutination (BIHA) titre
Cysticercosis ELISA.
Viral studies Epstein-Barr virus antibody studies; hepatitis-B antigen and antibody studies; human T cell lymphotropic virus type I (HTLV-I) antibody studies.

Stool and urine examination and rectal mucosal biopsy

For detection of bilharzial ova

Cerebrospinal fluid (CSF) tests

Cell count; protein, globulin, glucose concentrations; bacterial culture
Serological tests for syphilis.
Antischistosomal antibody detection using an ELISA test
Cysticercosis ELISA.
Human T cell lymphotropic virus type I (HTLV-I) antibody studies.
Cryptococcal antigen tests
Culture for fungi and acid fast bacilli } in selected cases.
CSF IgG and albumin.
Isoelectric focusing to detect oligoclonal IgG bands.

Radiological studies

Radiographs of chest and spine.
Conventional myelography followed by computed tomography (GE 8000) of the spine.
In selected cases, CT scan of the head.

Brain (1991), **114**, 727–741

DEFICITS IN STRATEGY APPLICATION FOLLOWING FRONTAL LOBE DAMAGE IN MAN

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SUMMARY

A quantitative investigation of the ability to carry out a variety of cognitive tasks was performed in 3 patients who had sustained traumatic injuries which involved prefrontal structures. All 3 had severe difficulties in 2 tests which required them to carry out a number of fairly simple but open-ended tasks over a 15–30 min period. They typically spent too long on individual tasks. All patients scored well on tests of perception, language and intelligence and 2 performed well on a variety of other tests of frontal lobe function. Explanations for their difficulty on the multiple subgoal tasks in terms of memory or motivational problems could be excluded. It is argued that the problem arose from an inability to reactivate after a delay previously-generated intentions when they are not directly signalled by the stimulus situation

INTRODUCTION

It has been known for many years that frontal lobe lesions can produce a gross effect in the performance of everyday life activities other than the most routine, even though neuropsychological tests suggest that the cognitive changes that have occurred are at most minor (e.g., Brickner, 1936; Ackerly and Benton, 1947). For instance, Eslinger and Damasio (1985) reported the case of an accountant who 6 yrs after an operation for the removal of a large bilateral orbitofrontal meningioma had an IQ of over 130 and performed well on a wide variety of neuropsychological tests, including some held to be sensitive to frontal lobe damage. Despite this excellent performance on quantitative tests, however, his ability to organize his life was grossly impaired. He was dismissed from a series of jobs even though his basic skills, manner and temper were appropriate. He went bankrupt and was involved in two divorces in 2 yrs. Relatively simple matters would take hours; thus to go out to dinner required that he consider the seating plan, menu, atmosphere and management of each restaurant and he might even drive to see how busy each of them was, but was still unable to come to a decision.

Of course, patients with relatively severe frontal lesions can have deficits on a range of quantitative neuropsychological tests (*see* Stuss and Benson, 1986, for review). It is possible that all patients who have debilitating everyday life difficulties would also show severe quantitative deficits on standard neuropsychological tests, but that the relevant tests were not given to the patient. However, a more probable explanation is that certain of the implicit approaches adopted in the design of neuropsychological tests makes the patient's impairment less likely to manifest themselves in the test situation. Thus in neuropsychological tests the patient typically has a single explicit problem to tackle at any one time, the trials tend to be very short (1 min or so or even less), task initiation

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is strongly prompted by the examiner and what constitutes successful trial completion is clearly characterized. Rarely are patients required to organize or plan their behaviour over longer time periods, or to set priorities in the face of two or more competing tasks. Yet it is these sorts of 'executive' abilities which are a large component of many everyday activities.

For instance, consider a situation which gave problems for a patient who had had a right frontal glioma removed and who was Wilder Penfield's sister. In Penfield and Evans (1935) he writes: 'She had planned to get a simple supper for one guest (Penfield) and four members of her family. She looked forward to it with pleasure and had the whole day for preparation. When the appointed hour arrived she was in the kitchen, the food was all there, one or two things were on the stove, but the salad was not ready, the meat had not been started and she was distressed and confused by her long continued effort alone.' If in addition to the actual preparation of the meal one also has to decide the menu, then an everyday task such as this will require certain capacities, none of which are clearly captured in the standard neuropsychological test. Planning will have to be carried out and preparatory purchases made. Different considerations—costs, tastes, available time, etc.—may have to be weighed against each other. Many minor decisions will need to be made and typically they are undertaken in parallel with other activities. There is no clearly correct solution and many different activities may have to be scheduled over the time available.

Before this second explanation can even be seriously considered, however, it is necessary to develop quantifiable analogues of the open-ended multiple subgoal situations where this subset of frontal patients would theoretically have problems. Only then can this possibility be realistically considered. In this paper we describe 3 patients with severe problems in the organization of everyday life activities arising from head injuries primarily affecting the frontal lobes. Their performance on a large set of standard neuropsychological tests ranged from generally good with patchiness on some 'frontal' tests in the worst of the 3 patients, through to almost consistently bright average to superior performance in the best of the 3. However, all 3 patients performed especially poorly on two tests designed to assess performance in more open-ended multiple subgoal situations. Various alternative explanations for their poor performance are then assessed.

CASE HISTORIES

Case 1

A.P., a right-handed man was involved in a road traffic accident at the age of 23 yrs, approximately 4.5 yrs before the present investigation. He sustained a serious open head injury involving an anterior fossa fracture, requiring multiple operations. His condition gradually improved, but he was not able to return successfully to his job. A CT scan that year showed evidence of extensive bifrontal damage. A year later he was admitted as a day patient to St Andrew's Hospital for rehabilitation. He was well-motivated and keen but could not carry out even the simplest activity because of an inability to keep his mind on the task in hand. For example, on one occasion he was discovered on the local golf course having originally stepped outside the therapy room to fetch some coffee. At these times he maintained that he 'completely forgot' whatever it was that he was supposed to be doing. He was unable to shop for himself because he would buy one item at a time returning to his car after his every purchase.

Rehabilitation as a day patient had limited success, and after 3 months A P. was transferred to another rehabilitation centre as an inpatient. There he was treated with behavioural methods described by Wood and Burgess (1988) with a slow but progressive improvement in his ability to organize his activities of daily living. He remained in rehabilitation for approximately 1 yr and then returned home to live with his parents.

In response to a clinical interview, he said that before the accident his room was immaculate ('5/5 tidiness') but it is now untidy with 'hotchpotch piles of magazines' on the floor. He had had an efficient filing system but had abandoned it. Shopping, cleaning and laundry are done for him. His social life is very rarely organized in advance. When questioned he said he had nothing planned for the coming weekend, was unable to give any example of consciously organizing an activity beforehand.

Clinical neuropsychological investigations. A.P. was tested at St Andrew's Hospital and then 2 yrs later, at the National Hospital on a large range of neuropsychological tests. On almost all he performed very satisfactorily. At St Andrew's Hospital he obtained a Verbal IQ of 121 and a Performance IQ of 120, which corresponds well with the estimate of 124 for his premorbid IQ as measured by the NART. There was one exception—the Digit Span subtest where he scored only in the Dull Normal range; however, on a repeat testing 2 yrs later he scored in the Superior range on the subtest. His performance on other subtests was slightly better, which is probably a practice effect (*see* Table 1). His spontaneous speech was not aphasic and on all perceptual, language, arithmetic and memory tests carried out he performed satisfactorily (*see* Tables 1, 2). The one memory test where his performance was below average was the Petrides and Milner (1982) self-ordered pointing task which is most appropriately considered a frontal test. On the picture version he made 7 errors by comparison with a mean of 3 errors for their control subjects who were of comparable age. (However, he was within 1 SD of the mean of a posterior lesion control group (*see* Table 3).) On all of a sizeable group of other tests thought sensitive to frontal lesions he performed very satisfactorily (*see* Table 3)

TABLE 1 PERFORMANCE ON BASELINE TASKS

Wechsler Adult Intelligence Scale (WAIS) Subtest Age-Scaled Scores

	Cases				*Cases*		
Verbal	*1*	*2*	*3*		*1*	*2*	*3*
Arithmetic	13	14	15	Picture Completion	14	12	13
Similarities	15	14	15	Block Design	15	12	13
Digit Span	15	14	15	Picture Arrangement	13	11	11
Vocabulary	16	15	17				
Verbal IQ	128	126	135	Performance IQ	129	112	114

	Cases			
	1	*2*	*3*	
WAIS Full Scale IQ	130	121	127	(Wechsler, 1955)
NART FSIQ equivalent	124	119	127	(Nelson and O'Connell, 1978)
Language (Scaled Score)	*A.P*	*D.N*	*F.S*	
Graded Naming Test	14	14	14	(McKenna and Warrington, 1983)
Perception (percentiles)	*A.P.*	*D.N.*	*F.S.*	
Cube Analysis	50	50	50	(Warrington and James, 1988)
Dot Centre	50	50	50	(Warrington and James, 1988)
Unconventional Views	50	25	10	(Warrington and Taylor, 1973)
Conventional Views	50	50	50	(Warrington and Taylor, 1973)

Case 2

D.N. a right-handed professional man, had sustained a serious head injury in a road traffic accident at the age of 26 yrs, involving a right frontal depressed skull fracture and an intracerebral haematoma treated surgically. He still has severe left hemiparesis. He says that consciousness was reduced or altered for 3 months. A CT scan carried out at age 48 yrs showed an extensive low attenuation area in the right frontal lobe and marked local atrophy of the right medial insular cortex. Both lateral ventricles were enlarged, the right more than the left. There were additional mild changes in the left frontal lobe. On leaving hospital after a stay of 6 months he returned to his previous employment but was unable to carry it out. He failed a higher degree but obtained a teacher's certificate. For the next 5 yrs he held a succession of jobs from most of which he was dismissed.

His responses in a clinical interview and his wife's testimony were in agreement over his domestic behaviour. He is untidy. He only bathes if going somewhere important. Shaving, changing his clothes or undergarments, washing his hair and having his hair cut are only carried out when his wife tells him. He hardly ever spontaneously tackles any domestic chores such a laundry, cleaning, cooking, making repairs or paying bills. If his wife is out he normally leaves the preparation of a meal to his 10-yr-old son. When he shops he never makes out a list himself and also usually comes home without all the items on the list his wife prepares. When she gives him a task she has to specify exactly what is required and even so he might carry out some parts only and then starts reading a newspaper. In addition, his wife organizes all trips, outings and social contacts with relatives. His wife said that he was occasionally irresponsible over money; for instance, even though they were in financial difficulties as he was out of work, he would buy gadgets they did not really need, including a sophisticated music system, costing £500.

Clinical neuropsychological tests. D.N. was tested in the National Hospital on a wide range of neuropsychological tests. On the WAIS, he obtained a Verbal IQ of 126 and a Performance IQ of 112, which may well be a little below his premorbid level but in fact corresponds well with the results of the National Adult Reading Test (Nelson, 1983) of 119. For all Verbal subtests he was in the superior range and in the Performance subtests in the average or bright average ranges (*see* Table 1). On memory tests he performed well with verbal material but poorly with visual material (*see* Table 2). On tests held to be sensitive to frontal lobe lesions his performance was generally satisfactory, but it was rather poor on the picture version of the Self-Ordered Memory test (*see* Table 3).

In summary, D.N. performed well on a wide range of perceptual language and frontal lobe tests. However, while his performance on verbal memory tests was well within the normal range, it was impaired on visual memory tasks.

Case 3

F.S., a 55-yr-old right-handed woman, employed in an undemanding post, as a 'media resources officer', had earlier sustained two separate head injuries. Thirty years before she had been thrown from a horse, had fractured her skull and had been left with a posttraumatic amnesia of unknown length. She was also aphasic at the time. The accident had left her with slight left-sided facial paralysis. Two years before being tested she had been knocked off her bicycle by a car and hit her head on the road; she did not lose consciousness but as a result has had a permanent loss of her sense of smell.

A CT scan carried out 2 yrs after the second accident showed an extensive lesion to the left frontal lobe with atrophy causing enlargement of the frontal horn of the lateral ventricle. There was also some atrophy in the left temporal lobe.

For the past 25 yrs she has worked in the same position. She lives by herself in a single room. Her responses in a clinical interview show that she undertakes virtually no inessential or novel activities. She is very untidy, never putting things away. She seldom goes out in the evening, and virtually never travels away from her home town. Others always make arrangements when any joint activity is to be carried out. She is said by her sister never to organize anything. She shops every day buying only a few things on any occasion and never visits supermarkets. She had no activity planned for the following weekend and could give no example where anyone had relied on her to do anything. Her sister confirmed that these behaviours were characteristic.

Clinical neuropsychological tests. F.S. was tested at the National Hospital on a large range of neuropsychological tests. On the WAIS she obtained a Verbal IQ of 135 and a Performance IQ of 114 comparable with the estimate of the premorbid IQ of 127 derived from the New Adult Reading Test. Verbal

TABLE 2 PERFORMANCE ON TESTS OF MEMORY (SCALED SCORES)

Test	*Cases* 1	2	3
Auditory Verbal Learning[1]	12[2]	8	9
Complex Figure Recall[1]			
Immediate	NT*	NT	6
Delayed	17[3]	3[3]	6
Digit Span[4]	15	15	15
Paired Associates (Sum)[5]	11	NT	7
Recognition Memory[6]			
Words	10	10	15
Faces	14	4	11
Story Recall[1]			
Immediate	13[5]	11	11
Delayed	14[5]	10	12
Visual Reproduction[5]	13	14	NT

* NT = not tested [1] Coughlan and Hollows (1985) [2] Score on Worst Trial of Rey Auditory Verbal Learning Test (Rey, 1964). [3] Osterrieth (1944–1945). [4] Wechsler (1955) [5] Wechsler (1945) [6] Warrington (1984).

TABLE 3 PERFORMANCE ON 'FRONTAL LOBE' TASKS

	Cases 1	2	3	*Comment*
Alternation task[1] (trial of last error)	90%ile	75%ile	5%ile	Compared with nonlesion patient/controls[2]
Bilateral hand movements	Good	Good	Good	
Cognitive Estimates[3] (error score)	2	4	5	Mean of controls = 3.0, SD 2.6[2]
Money's Road-Map Test[4] (errors)	0	3	5	Mean of left frontal group = 11 9, normals = 1.7
Personal Orientation Test[5] (errors)	0	4	14*	Mean of right posteriors = 4.1, SD = 3.5 Mean of left posteriors = 2 4, SD = 2 7[6]
Proverb Interpretation	Good	Good	Good	
Self-Ordered Memory[7] Representational Pictures (errors)	7	10	9	Cases 2 and 3 >1 SD worse than a lesion control group[8] (mean = 5 9, SD 2.8)
Stroop[9] (time)	20 1	17.3	21.0	Control mean = 23 3, SD = 11[2]
Tower of London (score)[10]	33	23	24	Controls. mean = 24.9, SD = 4.5[2]
Trail Making Letters and Numbers[11] (completion time in s)	15 7	25.6	49.0*	Controls· mean = 29.6 s, SD = 4 5[2]
Verbal Fluency· Letters FAS, each 60 s (total retrieved)	70	34	33	Controls mean = 34 8, SD = 14 3 (n = 30)[12]
Modified Wisconsin[13] (categories achieved)	6	6	6	Controls mean = 5, SD 1 6
Total errors	3	3	1	Controls mean = 9 2, SD 8.5 (n = 46, mean age (yrs) 45, SD 14)

* More than 2 SD worse than controls [1] Chorover and Cole (1966). [2] Nonlesion patient controls (n = 24, mean FSIQ = 114.9, SD 12.7). Shallice, Warrington, Watson and Lewis (unpublished study) [3] Shallice and Evans (1978) [4] Butters *et al* (1972). [5] Semmes *et al* (1963). [6] 10 right posterior lesion patients (mean FSIQ 108.2, SD 9 8, mean age 45.7 yrs, SD 14 0) and 10 left posterior lesion patients (mean FSIQ 108 1, SD 12.2, mean age 41 8 yrs, SD 14.0). [7] Petrides and Milner (1982). [8] 8 right posterior patients (mean FSIQ = 107 1, SD 8 9, mean age 45.5 yrs, SD 14.8. Mean Scaled Score FC Faces = 7 25, SD 4.8 (dull average)). [9] Perret (1974). [10] Shallice and McCarthy (*see* Shallice, 1982). [11] Reitan (1958). [12] Miller (1984). Note, however, that if Miller's formula for predicting fluency from WAIS verbal subtests is used, Cases 2 and 3 both fall below the expected range [13] Nelson (1976).

subtests were all carried out at the Superior level. Visual subtests were carried out at the Bright Normal level except for Picture Arrangement which was at the average level. She had no aphasic difficulties and performed in the normal range on language and perceptual tests (*see* Table 1)

Her performance on memory tests was mixed (*see* Table 2). However her pattern of spared/impaired performance, with difficulty on learning span +1, which she was unable to do in 12 trials for both digits and spatial positions (Corsi span (*see* Milner, 1971)); also on the Rey Figure, in the context of spared Recognition Memory, Logical Memory and free recall suggests that she has a memory problem secondary to frontal difficulties (Signoret and Lhermitte, 1976).

On tests thought sensitive to frontal lobe damage her performance was variable (*see* Table 3). On a number it was satisfactory, but on 4 tests it was impaired. Thus both memory and cognitive tasks showed evidence of frontal impairment.

Summary of patients

Overall the 3 patients perform well and close to their premorbid level on a wide range of perceptual, language and general cognitive tests. Case 1 has no problem with memory tasks but Case 2 has a primary visual memory impairment and Case 3 has difficulties on certain tasks of a 'frontal memory' type. Two of the patients perform very well on a wide range of tasks thought sensitive to frontal lobe lesions but 1 shows impairments on some frontal tasks. All show severe organizational difficulties in everyday life.

NEUROPSYCHOLOGICAL TESTS

Multiple subgoal scheduling tests

Two tests were developed to measure the ability of a subject to schedule the performance of a number of simple tasks when the demands made are rather open-ended and there are a number of simple rules which apply across all tasks.

The Six Element Test (SE). The purpose of this test, which is undertaken in a standard hospital office, is to assess the ability of the patients to carry out 6 open-ended tasks in a fixed period of time (15 min) in a way that maximizes their overall score. The 6 tasks divide into 2 sets of 3—dictating a route, carrying out arithmetic problems and writing down the names of approximately 100 pictures of objects. Each set of arithmetic problems contains 35 problems generally of increasing difficulty. Thus in set A,

$$\text{no. 8 is } \tfrac{300}{6}\times 4,\ \text{no. 18 is } 201+187-86 \text{ and no. 28 is } \tfrac{210}{7}\times 8+(6\times 4).$$

The 2 sets are equivalent in difficulty.

The subject is presented with the instructions written on a card (*see* Appendix 1). The examiner (E) first reads through the complete test instructions with the subject (S), explaining which stacks of cards are piles A and B and which arithmetic set is I and II and so forth. Having read through the task descriptions the E says 'But before you go on, there are a number of important rules you *must* follow which govern the way you are allowed to do these tasks, and may influence the way in which you decide to organize the way you do the test'. The 5 rules are read through with the S and explained as fully as necessary. The instruction sheet is then turned over and the S is 'tested' on his recall of the rules. Any errors are corrected. If she/he has forgotten any, the entire rules are read through and then the S is tested again. This procedure is repeated until all the rules are recited correctly. It is explained to the S that the instructions will be left in front of them at all times to consult at will.

Two subsidiary tests were also undertaken. First, the subjects, whose watches had been removed, had to carry out the Digit Symbol subtest of the WAIS, stopping themselves after 1 min 15 s. A stop watch was visible throughout. Secondly, Rey's (1964) Tangled Lines test had to be carried out with the subjects instructed to stop as close as possible to 2 min 30 s after starting. This time the stop watch was covered by a small cardboard box. The box could be moved whenever the subjects wanted to know the time but had to be immediately replaced. With both these tests, were the Ss not to stop themselves at the appropriate time the E made no indication that they were to stop, and they were allowed to continue.

Controls. 10 control subjects were tested. They were matched to the patients on age (mean 41, range 25−62 yrs), educational background, and NART IQ (mean 120.2, range 113−127 yrs).

TABLE 4 PERFORMANCE ON THE SIX ELEMENT TASK

Case	*No. of subtasks tackled*	*Max. time on any subtask (min, s)*
1 (I)	2	7,30
1 (II)	4	6,19
2 (I)	5	10,11
2 (II)	3(+2 incorrect)	6,22
3 (I)	3	7,18
3 (II)	2	9,30
Controls	5.7 (±0 5)	5,35 (±0,53)

Results. Two measures of task performance are shown in Table 4. Two different attempts were made by each patient, at least 3 wks apart. On the first attempt Cases 1 and 3 tackled less subtasks than any control and all 3 spent more time on their subtask they attempted for longest (*see* Table 4).

Qualitatively the behaviour of the patients was also atypical. On his first attempt A.P. made notes for over 4 min to help with the dictation task, but in fact never dictated at all. He only attempted 2 subtasks, the second of these occupying the last 7.5 min. On his second attempt he tackled only 4 of the subtasks claiming that he did not dictate his journey back as he had not decided where he would go when he would leave the hospital! Case 2 succeeded in tackling 5 subtasks but spent much longer than any normal subject—over 10 min in 3 separate periods—on 1 of the subtasks (maths I) without ever trying its complementary task (maths II). On his second attempt he behaved bizarrely, changing task 63 times (mean task changes of controls 5.7, SD 2.19) and dictating the two sets of picture names—spending only 14 s and 20 s on them—instead of writing them down; no normal subject carried out a subtask incorrectly. Also on 6 occasions he carried out a subtask immediately after the complementary one, so breaking one of the rules. (One control also made this error. She said she had mistaken the rule as not doing a part I of a pair of subtasks after another part I; this she correctly applied.) Case 3, on her first attempt, only tackled 3 subtasks. On the second she wrote her journey instead of dictating it, attempted only 2 other subtasks and spent the last 9 min on just 1 of them. During this time she looked at the stopwatch on 7 occasions but did not switch tasks.

On the 2 timing tasks Case 1 performed well on both (1.5 min, stopped 1 min 17 s; 2.5 min, stopped 2 min 28 s). Case 2, however, failed the first, stopping at 1 min 38 s although being fully aware he had to stop at 1 min 15 s. On the second task his performance was very poor on both testing occasions. On the first attempt he stopped after 1 min 37 s, saying that he thought 2 min 45 s had elapsed and on his second he failed to stop until 3 min 26 s had elapsed. Case 3 performed well on the 1.25 min task (stopping at 1 min 15 s). However, on the more difficult 2.5 min task she did not stop until 3 min 20 s had passed, despite having looked at the watch at 2 min 38 s. No control was more than 1 s out on the first task or more than 5 s out on the second. (Mean time out for task 1, 0.1 s, SD 0.3; task 2, mean 2.9 s, SD 2.0).

Comment

All 3 patients performed at below the normal range on quantitative measures of performance on the Six Element task. In addition their performance was also qualitatively atypical.

Multiple Errands Test (ME). The purpose of this test, which is undertaken in a pedestrian precinct near the hospital previously unknown to the patients, is for them to carry out a number of tasks in situations where minor unforeseen events can occur. The subtasks the patients had to carry out are basically very simple except for one, which has subcomponents designed to be reasonably demanding for someone of the IQ level and cultural background of the patients. While still inside the hospital the patient is given a card with 8 tasks written on it, 6 of which are simple (e.g., buy a brown loaf, buy a packet of throat pastilles). A seventh requires the subject to be at a certain place 15 min after starting. An eighth is more demanding, 4 sets of information have to be obtained and written on a postcard, namely: (1) the name of the shop in the street likely to have the most expensive item; (2) the price of a pound of tomatoes; (3) the name of the coldest place in Britain yesterday; and (4) the rate of the exchange of the French franc yesterday.

The card also contains the following instructions: 'You are to spend as little money as possible (within reason) and take as little time as possible (without rushing excessively). No shop should be entered other than to buy something. Please tell one or other of us when you leave a shop what you have bought. You are not to use anything not bought on the street (other than a watch) to assist you You may do the tasks in any order.'

This second part of the instructions—the 'rules'—is then read aloud to the patient who is asked to repeat them. If the rules cannot all be repeated satisfactorily the procedure is repeated until they can be. The subject is then taken the short distance to a shopping precinct where he or she is asked to repeat the rules again. If there is any failure to reproduce the gist of any instruction the subject is reminded of it. It is then indicated to the patient where the limits of the test area are; they are very clearly demarcated by a large street ending in the pedestrian precinct at one end and a set of traffic lights at the other. The behaviour of the subjects while carrying out the activity is monitored by 2 observers and they are also debriefed after the task had been completed.

Controls. Nine control subjects were tested. They were matched with the patients for age (mean 40, range 24–63 yrs) and NART IQ (mean 122, range 113–127).

Results. Table 5 gives the number of errors produced by each of the 3 patients. Each produced at least 2 SDs more than the controls. Table 5 also shows the errors broken down into subcategories: (1) *inefficiencies*—where a more effective strategy could have been applied, e.g., entering the same shop more than once; (2) *rule breaks*—where a specific rule (either social or explicitly mentioned in the task) is broken, e.g., going outside the boundaries or leaving a shop with a newspaper without paying; (3) *interpretation failure*—where the requirements of a particular task are misunderstood, e.g., assuming that the information must be written on the birthday card rather than the postcard; (4) *task failure*—a task either not carried out or not completed satisfactorily.

TABLE 5 ERRORS ON MULTIPLE ERRANDS

	Cases			
	1	*2*	*3*	*Control*
Inefficiencies	6*	9*	5*	1 4 (1.1)
Rule breaks	5*	8*	8*	1 6 (1 3)
Interpretation failures	1	1	1	0 4 (0 7)
Task failures	0	5*	4*	1 1 (1 4)
Total errors	12*	23*	17*	4 6 (2 1)

* More than 2 SD worse than the control subjects.

All 3 patients performed at the 5% level or worse, as estimated from the control data, on both the number of inefficiencies and on the number of rule breaks. There was no difference overall in the rate of errors of the other two types, although Case 3 produced more task failures. Thus 2 normal controls as well as the patients failed to provide the required information on the postcard, probably because the information required was placed on the *back* of the instruction sheet.

Qualitatively the performance of the patients was even worse. They made types of error which were not produced by any control. Case 3 used a clearly irrelevant criterion inappropriately when she broke a rule (entering a shop without buying anything) because when in the shop (a chemist) she found it did not have a soap she especially liked; other cheap soap—which would have been at least as adequate for the task— was available. She also failed to note the time when starting. Cases 1 and 2 both became involved in social complications. Case 1 made the interpretative error of deciding he needed yesterday's newspaper (to find the coldest place in Britain on the previous day). He entered a newsagents and asked if they had the previous day's paper. They had, so he walked out with it, incidentally breaking the buying rule. He was pursued into the street by the shopkeeper who wanted to be paid for it. A.P. had just assumed that because a previous day's newspapers are generally worthless he could have one without paying. Case 2 produced a complex set of errors. Thus one series of actions began with his being the only subject to enter a shop simply to ask about the location of another type of shop (breaking buying rule). He was referred to a shop outside the allowed area, went to that shop (breaking the limit rule) and became involved in a heated argument with a shop assistant as he asked her to give him a birthday card free (which would have also broken the buying rule)!

Comment

As with the Six Element task, all 3 patients had quantitatively impaired performance, in particular as far as rule breaks and inefficiencies were concerned. Their performance was also qualitatively unusual.

DISCUSSION

We have described 3 patients, each of whom had sustained head injuries in which the area of focal damage lay in frontal regions. The 3 patients performed well on those cognitive tests which were not designed to load specifically on 'frontal functions'. On 'frontal' tests their performance varied. Case 3 performed poorly on a number of such tests. A second (Case 2) performed generally satisfactorily except for a borderline impairment on the Petrides and Milner Self-Ordered Memory. The third (Case 1) performed very satisfactorily on all standard 'frontal' tests. In daily life, however, all 3 appeared unable to act effectively on their own initiative as they organized virtually no nonroutine activities. In addition 2 (Cases 1, 2) were reported to have problems in carrying out multiple subgoal tasks such as shopping and the third (Case 3) avoided such activities.

These difficulties were mirrored in the 2 tests where they had to perform multiple activities, the Six Element Test and Multiple Errands. The 2 tasks required the subject to carry out a number of fairly simple activities in an efficient manner. One, the Six Element, basically involved the devising of a simple plan, scheduling the subtests efficiently and keeping a check on time. The second involved dovetailing the activities in a more real-life setting where minor unforeseen events could occur. All 3 patients performed both quantitatively and qualitatively worse than the normal controls.

If one considers what is involved in carrying out these multiple subgoal tasks, then at a very general level, four basic types of process are relevant. Motivational and memory processes are clearly required and so are a variety of special-purpose cognitive processes of the sort that standard neuropsychological tests assess. In addition there are certain bridge processes which enable the special-purpose cognitive processes to be used to satisfy motivational requirements. A deficit in basic special-purpose cognitive processes seems an implausible explanation of their difficulties on the experimental tasks, given the performance of the patients on the baseline tests. Indeed the most difficult Multiple Errands subtest gave problems for some of the controls as well as the patients; it was the least sensitive part of the procedure. However, frontal patients often manifest inappropriate affect and have frequently been described as apathetic or impulsive (Blumer and Benson, 1975), and also they can have memory problems (e.g., Hécaen, 1964). The possibility of motivational or memory difficulties therefore needs to be considered, especially as the patients, when asked to account for some action, often said that they had completely forgotten their prior intention.

A possible motivational explanation of the impaired performance of the 3 patients is that they require continuous social reinforcement to carry out psychological tasks, and without it their spontaneous motivation would tend to dwindle rapidly; without it they do not persevere. This account is unsatisfactory. Case 1 was given the entire AH6 followed by the Watson-Glaser Critical Thinking Appraisal (Watson and Glaser, 1980) and left to work through them on his own. He scored at the 50th and 80th percentiles, respectively, the total of the task times being 2 h. Both Cases 2 and 3 carried out the long form of Raven's Matrices working through the test on their own. This took them 31 and 42 min, respectively—less than the 45 min that Raven *et al.* (1977) give as the upper limit of the normal range and yet longer than the 2 multiple-subgoal scheduling tests would normally take. They scored at above the 95th and 80th percentiles, respectively, for their ages on the Matrices.

For the Six Element task a motivational explanation has in any case little immediate face validity. It requires little effort to switch from one simple task to another. More critically, Cases 2 and 3, the subjects for whom a motivational explanation is most plausible on clinical grounds, made errors analogous to the ones made on the Six Element task on an even simpler timing task. When they had to stop carrying out a perceptuomotor task after 2.5 min with no external timing signal but a stopwatch available, they continued after the time limit. Lack of perseverance—the motivational explanation being considered—should presumably not lead to overshoot! Thus motivational factors do not seem likely to be central to their deficit.

Standard retrospective memory processes are also relevant, at least for carrying out the Multiple Errands test where retaining both the layout of the street and the instructions, without using the card, would be helpful. The patients differed in this respect. Case 1 had no difficulties. Case 2 was entirely normal on verbal memory tasks but had visual memory problems which might have affected his performance of the Multiple Errands test; however, they would seem most unlikely to contribute to his difficulty with the Six Element task where there is no spatial memory load and where the instructions are continuously clearly visible on the desk. Case 3 had problems on some memory tasks but these were all in tasks where active organization of the input is required for adequate memory performance and her difficulties appear secondary to her frontal lobe problems (*see* Signoret and Lhermitte, 1976). She was able to carry out memory tasks where the organization was provided for her and in particular she retained text normally, as indicated by her satisfactory performance on Logical Memory where the amount she retained showed no decline over an hour's delay. Like the other patients, she was able to recite correctly the instructions for the tasks in her own words after they had been presented to her. This makes it most unlikely that retrospective memory difficulties lie at the root of her problems, at least on the Six Element test.

Given that the problems these 3 patients exhibit on these multiple subgoal tests do not fit well with an assumption of basic motivational impairments on the one hand or of special purpose cognitive or retrospective memory processes on the other, do they fit with impairments to the bridge processes that lie between the two? To assess this it is necessary to consider the bridge processes in more detail.

These processes lie in the domain of the creation and maintenance of goals and intentions, of their realization at appropriate times (prospective memory (Meacham and Leiman, 1982; Kvavilashvili, 1987)) and of planning. This is an area where well developed theories are lacking. However, a position developed by one of us in collaboration with D. A. Norman is that where a task cannot be adequately carried out through the application of well-learned action or thought routines alone, it requires the use of a Supervisory System, which is anteriorly located in the cortex (Norman and Shallice, 1980, 1986; *see also* Shallice, 1988). We will provisionally follow this approach and assume in line with it that the performance of a basic routine activity, like paying the assistant when shopping, is not dependent on anterior systems once it has been selected for operation. What processes would a Supervisory System control?

It is often argued that when routine actions will not achieve a goal satisfactorily the problem-solving process required involves at least four stages and that these may be called in a recursive fashion (de Groot, 1965; Luria, 1966; Ben-Yishay and Diller, 1983). One or more potentially achievable goals must be specified: a course of action (a plan) must be drawn up, a solution attempt is then executed and finally the provisional solution assessed.

It is now argued that in normal human activity plans when originally developed do not correspond to completely worked out courses of action (Suchman, 1987; Ellis, 1989). A consequence is that planning as a whole needs to be viewed as a more opportunistic process carried out on-line when opportunities arise or difficulties occur. There must, however, be processes related to intentions, which link the schematic plan with the potential to realize it effectively at some later time. We propose that these processes operate through the activating of markers and their later triggering to interrupt ongoing behaviour at a future time. A marker is basically a message that some future behaviour or event should not be treated as routine and instead, some particular aspect of the situation should be viewed as especially relevant for action. If the behaviour or event does occur later, then the marker would be triggered and this would lead to inhibition of the activity being carried out, the reassessment of the situation and so potentially the switching in or out of a particular course of action linked to the marker. Thus a task rule, such as not entering a shop unless something is to be bought, would be realized through a marker becoming activated when the instructions are understood, that when about to enter a shop a check should be made that something is to be bought. This marker would then be triggered by the action of approaching a shop or thinking of doing so. Many markers could be simultaneously activated.

On this view the following processes are plausibly controlled by a Supervisory System, although all are also likely to involve other systems (1) goal articulation; (2) provisional plan formulation; (3) marker creation; (4) marker triggering (by an appropriate mental or physical event). In addition, an evaluation process can occur at any stage and if the result is that the present situation or solution is unsatisfactory this would lead to articulation

TABLE 6 SIMPLE SCENARIO TO ILLUSTRATE COMPONENT PROCESSES IN PRACTICAL PROBLEM SOLUTION

7 45 a m	Situation· receive urgent letter.
7.50 a m	Goal Articulation. to reply as soon as possible (NB, no stamp available).
8.00 a m	Plan formulation decide to buy stamps at Post Office on way to work and write letter immediately on arrival and post it at lunchtime.
	Marker Creation 1—When leaving underground → Go to P.O.
	Marker Creation 2—When arrive work → Write brief letter (if not possible on arrival—carry out Plan Modification).
	Marker Creation 3—When go to lunch → Post letter
9.00 a m	(Leave Underground) Marker Trigger 1 → Go to P O
9.15 a m	(Arrival work) Marker Trigger 2 → Write letter*
12.30 a m	(Go lunch) Marker Trigger 3 → Post letter

* If not possible to write letter at that time plan modification occurs and new markers set up.

of unforeseen subgoals and hence plan modification; this would also be necessary if unforeseen events occur. These stages are illustrated in the simple scenario shown in Table 6.

The present 2 multiple-subgoal tests appear to require all these processes and many of the errors that occur in the execution of the tests appear to occur from a failure of one or other of them, although quite frequently it is not possible to be precise about which process is impaired. However, if one groups the above processes into three types then a provisional grouping of the lapses that occur in the 2 experimental tasks can be made, although possible overlaps between the three types still exist. The types are: (1) Plan Formulation or Modification; (2) Marker Creation or Triggering; (3) Evaluation and Goal Articulation (*see* Table 6 for an example). Table 7 gives examples from the 2 experimental tasks of the three types of lapse. It can be seen that there are examples of all three types being made by each of the patients, but the prevalence of rule breaks in the behaviour of all 3 patients suggest that marker errors are a particularly important problem. It is also apparent that the marker errors can be subdivided. In some (1, 5) the marker has to respond to a temporal cue, in some to a social convention cue (3) and in some to a task rule cue (2, 4). In all these cases triggering the marker should lead to the inhibition of the ongoing behaviour, but in other cases triggering the marker should have led to a positive behaviour. However, for this type of error there was much more overlap with control subjects (e.g., not coming to tell E when something was bought in a shop (all 3 patients, but also 5/9 controls)).

TABLE 7 EXAMPLE OF THE THREE TYPES OF LAPSE MADE BY THE PATIENTS ON THE EXPERIMENTAL TASKS

Plan formulation or modification	*Marker creation or triggering*
1. Going to Post Office before all relevant information obtained (Cases 1–3)	1. A very extended period spent on a single SE subtask without a break (Cases 1,3)
2 No plan for SE (Cases 1–3)	2. Going out of bounds in ME (Cases 2, 3)
3. Failure to look at watch at start of ME (Case 3)	3. Attempt not to pay for item in shop (Case 1, 2)
4 Not knowing quite how he would get to X and therefore not tackling SE. Journey back subtask (Case 1)	4. Looking in irrelevant shops (Cases 1, 2)
5 Generation of inappropriate criteria in an ME subtask (Case 3)	5. Not using the notes made for 4 min about journey at start of SE

Evaluation and goal articulation

1. Posting postcard without all information (Cases 2, 3)
2. Failure to keep a check of money in ME (Cases 2, 3)
3. Thinking finished ME when had not (Case 1)
4. Ending SE attempt 1 min early when far from having completed subtasks (Case 3)
5 Carrying out SE subtasks incorrectly (Cases 2, 3)

SE = Six Elements; ME = Multiple Errands

If the everyday life behaviour of the patients is examined, it is often less easy to categorize many of the lapses that they make because they involve a larger period of time. Nevertheless, the problems they manifest seem likely to arise from the same source as their difficulties in the experimental task.

The processes of plan formulation and evaluation have often been ascribed to frontal lobe systems (Penfield and Evans, 1935; Luria, 1966) as has goal articulation (Duncan, 1986) and together with marker creation and triggering, these processes are key elements in the temporal integration of behaviour which Fuster (1980) argued to be the basic function of the frontal lobes. To our knowledge, however, no experimental setting has been investigated in which errors occurred that could be clearly ascribed to impairments in these processes in patients whose other cognitive functions were shown to be essentially intact. If the errors of marker creation or triggering are considered, the failure to interrupt ongoing behaviour and produce a specifically relevant action is related to the difficulty of inhibiting central sets ascribed to frontal systems by Rosvold and Mishkin (1961), but the effects of marker creation and triggering need not all be inhibitory in nature.

Overall, plan formulation/modification, marker creation and triggering and evaluation and goal articulation may be considered complementary component processes undertaken by the Supervisory System on the model of Norman and Shallice (1980), and it is these processes that are impaired in the Strategy Application Disorder. Whether it is possible for the individual components to be selectively impaired is not possible to assess with the current evidence. However, in Case 1, marker creation and triggering processes seemed the most affected, while in Case 3 it was goal articulation, plan formulation and evaluation. It therefore seems a plausible possibility that finer fractionation of component processes undertaken by the Supervisory System can occur.

ACKNOWLEDGEMENTS

We would like to thank Mr J. R. Bartlett, Drs C. J. Earl, P. Lloyd, M. Rose and C. M. Wiles for permission to publish information on patients at present or formerly in their care. We are very grateful to Professor Elizabeth Warrington for providing facilities for the research to be carried out. We also wish to thank Dr R. Ll. Wood for his advice and assistance, and Dr Antonio Damasio for his helpful comments on an initial version of the paper.

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(Received October 20, 1989. Revised March 30, 1990. Accepted April 17, 1990)

APPENDIX. SIX ELEMENT TASK INSTRUCTIONS

In the next 15 min we would like you to carry out 3 different tasks, each of which is in 2 parts. The tasks are:

1 To dictate* into the tape-recorder a *brief* account of two journeys (a) Your journey to here; (b) your intended journey from here.

2 To write down the names of as many pictures as you can (in order) (a) Those in the left pile (set A), (b) those in the right pile (set B).

3. To solve 2 sets of arithmetic problems (as many as you can, in order). (a) Set I; (b) set II

But you are not allowed to do the 2 subtasks (a) and (b) of the same type one after the other. Each of the 6 subtasks is given equal weight. Within subtasks 2 and 3, points will be given for correct answers Earlier pictures/problems will be given more points than later ones in each subtask *and* errors/omissions will be penalized.

* The cassette recorder records throughout the 15 min so that the subject does not need to operate it

Brain (1991), **114**, 743–754

BASAL TEMPORAL LANGUAGE AREA

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SUMMARY

Language interference was elicited by electrical stimulation of the dominant basal temporal region in 8 out of 22 cases and in none of 7 cases with subdural electrodes implanted over the nondominant temporal lobe. Language interference was elicited by stimulation of electrodes placed over the fusiform gyrus 3–7 cm from the tip of the temporal lobe.

Electrical stimulation of the basal temporal language area produced a global receptive and expressive aphasia with speech arrest at high stimulus intensities. Other higher cortical function, for example copying complex designs or memory of nonverbal information was intact, in spite of the total inability to process verbal information. At lower stimulus intensities partial aphasias with a predominant receptive component occurred. Surgical resection of the basal temporal language area produces no lasting language deficit.

INTRODUCTION

Three cortical language areas were defined by Penfield and collaborators by electrical stimulation studies (Penfield and Rasmussen, 1949, 1950; Penfield and Jasper, 1954; Penfield and Roberts, 1959; Penfield and Perot, 1963). They described anterior, posterior and superior language areas which were located approximately at Broca's, Wernicke's and the supplementary motor area, respectively. The occurrence of language deficits during electrical stimulation of Broca's and Wernicke's areas were later widely confirmed by other investigators (Fedio and Van Buren, 1974; Rasmussen and Milner, 1975; Ojemann, 1978, 1979; Ojemann and Whitaker, 1978; Van Buren *et al.*, 1978; Ojemann and Mateer, 1979; Rapport *et al.*, 1983; Lesser *et al.*, 1984*a*, 1986). Electrical stimulation studies of the human supplementary motor cortex, however, have been performed only infrequently and there are no additional reports of language interference by stimulation in that area.

In 1984, we studied a patient who showed a clearly defined language deficit when stimulating the fusiform (occipitotemporal) gyrus of the dominant temporal lobe (Lüders *et al.*, 1985, 1986*a*). We now report on the results of stimulating the basal temporal region of 26 patients with intractable complex partial seizures who were being evaluated for surgical treatment of epilepsy.

MATERIAL AND METHODS

Twenty-nine patients had 16 or more subdural electrodes implanted over the basal temporal region for work-up for surgery for epilepsy between December 1983 and February 1986 (Lüders *et al.*, 1989). Some of the patients with the electrodes placed over the basal temporal region of the nondominant hemisphere

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were not included because no systematic stimulation studies were performed. Cases with less than 16 subdural electrodes over the basal temporal region were also excluded. The age of the patients ranged from 14 to 36 yrs, and they all had intractable complex partial seizures.

All patients had presurgical EEG/video monitoring during which the anticonvulsants were discontinued and interictal and ictal epileptiform activity was recorded from surface electrodes including closely placed scalp, periorbital, nasopharyngeal, ethmoidal, and sphenoidal electrodes (Lüders *et al.*, 1982; Lesser *et al.*, 1984*b*; Morris and Lüders, 1985). This evaluation lasted a minimum of 5 days but in selected cases up to 14 days. The information obtained from this work-up was then used to decide the type and site of insertion of the subdural electrodes. In addition, each patient had computerized tomography (CT) of the brain, cerebral angiography, dichotic testing, testing of visual fields and detailed speech and neuropsychological testing. Speech and language function was evaluated preoperatively through administration of the Boston Diagnostic Aphasic Examination, Boston Naming Test, Revised Token Test, Word Fluency Test, and assorted informal measures. This test battery was repeated at 6 months postoperatively. Magnetic resonance imaging of the brain was also performed in selected patients. Speech and short-term memory dominance was tested with intracarotid amylobarbital injections (Wada, 1949; Wada and Rasmussen, 1960).

The subdural plates consisted of stainless steel electrodes, 3 mm in diameter and 0.9 mm thick which were embedded in Silastic material with a centre-to-centre distance between the electrodes of 1 cm. These electrodes were custom made and details of their design and insertion technique have been published elsewhere (Lüders *et al.*, 1987). Twenty-one patients had only one 4×4 plate (16 electrodes) implanted under the basal temporal region. Three patients had 4×4 plates inserted under both basal temporal areas. Two patients had a 4×6 plate (24 electrodes) implanted under one basal temporal region. In addition, all patient had extensive coverage of other cortical areas with subdural electrodes. In most cases, this consisted of a 8×8 plate (64 electrodes) over the temporal lobe convexity and immediately adjacent suprasylvian areas. A typical example with a 4×4 plate over the basal temporal region and an 8×8 plate over the convexity is shown in fig. 1.

The subdural electrodes were inserted on a Thursday or Friday and, after giving the patient a short period to recover from the operation, stimulation and evoked potential studies were started the following Monday. These functional studies were in all cases completed in 1 wk. After discontinuing or decreasing

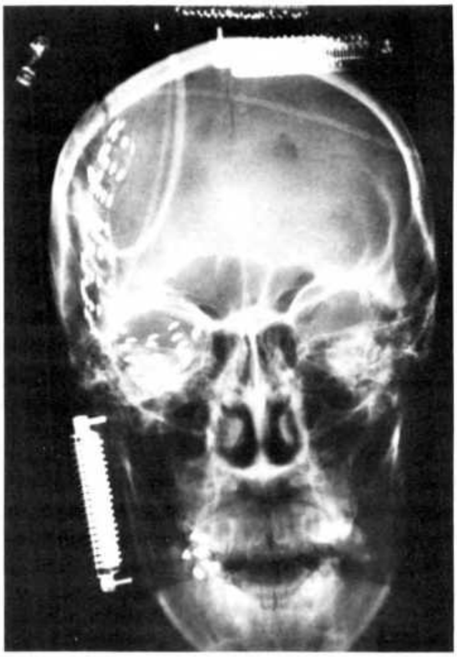

FIG. 1. X-ray of patient with a chronically implanted 4×5 plate of subdural electrodes over the basal temporal region and an 8×8 plate over the lateral convexity of the temporofronto-parietal region.

anticonvulsants, interictal and ictal epileptiform activity was recorded from the subdural electrodes for at least 5 days. Surgery consisted of excision of all cortical areas which had shown interictal and/or ictal epileptiform activity as long as they did not include functionally important areas as defined by the electrical stimulation and evoked potential studies. More details of this work-up strategy have been published elsewhere (Lesser *et al.*, 1984*b*; Lüders *et al.*, 1987).

The electrical stimulation studies included an initial screening test. All the electrodes were stimulated starting at an intensity of 1 mA. The stimulus duration was 0.3 ms, and 5 s duration trains of 50 Hz alternating polarity stimuli were delivered. The stimulus intensity was increased at 0.5 – 1 mA steps until (1) 'positive' symptoms occurred (muscle twitch, paresthaesiae, hallucinations), (2) after-discharges were elicited or (3) a maximum stimulus intensity of 15 mA was reached. During the screening stage stimulation was always 'referential' using as 'reference' an electrode which showed no after-discharges or 'positive' symptoms at an intensity of 15 mA. All the electrodes which did not show 'positive' symptoms were also screened for language disturbances by having the patient read aloud while stimulating at 0.5 mA below the after-discharge threshold (maximum of 15 mA if no after-discharges occurred). Stimulation intensities slightly below after-discharge threshold were also used to test for other higher cortical functions. These special tests were usually only performed at electrodes in which the 'reading aloud test' showed abnormalities. Details of the tests performed at these electrodes are given in the Results section.

RESULTS

Stimulation of the basal temporal region produced interference with language (speech arrest or slowing down during the 'reading aloud test') in 8 out of 22 cases with plates under the dominant temporal lobe but in none of the cases with plates under the nondominant temporal lobe. Speech arrest was noted during stimulation in at least 1 electrode in 7 out of 8 patients. These included 3 patients who had plates inserted in both sides but language interference was not elicited by electrical stimulation on either side.

When stimulating the prerolandic inferior frontal region (corresponding approximately to Broca's area), language interference was elicited in 15 out of 22 patients with plates over the dominant hemisphere but in none of the 7 patients with plates over the nondominant hemisphere.

When stimulating the superior temporal gyrus behind the point where the rolandic and sylvian fissures meet (corresponding approximately to Wernicke's area), language interference was elicited in 14 out of 22 patients with plates over the dominant hemisphere but in none of the 7 patients with plates over the nondominant hemisphere.

Interference with language was elicited at an average of 2.7 electrodes (range 1 – 15) at Broca's area, 3.6 electrodes (range 1 – 7) at the basal temporal area, and a 4.0 electrodes (range 1 – 16) at Wernicke's area.

In the 8 patients in which we were able to demonstrate the existence of a basal temporal speech area, stimulation while they were asked to read aloud produced either total speech arrest or marked slowing. One patient indicated that he 'was looking at the word but was unable to spit it out' or that he 'was able to see the word but could not read it'. Another patient described it 'as being able to see but not to read the word'. The use of different intensities of stimulation also showed that the degree of language interference was a direct function of the intensity of stimulation. For example, 1 patient at 4 mA of stimulus intensity could read with no difficulty; at 4.5 mA the reading became markedly slowed and at intensities of 5 – 10 mA complete speech arrest occurred.

The patients were also asked to perform rapid alternating movements of the tongue and/or fingers while stimulating the electrodes where speech arrest occurred. No interference of rapid alternating movements was observed.

In 3 patients, more detailed aphasia testing was carried out. All 3 were unable to read simple words during electrical stimulation. Two of them were unable to repeat the word after stimulation. The third patient, however, was able to identify the word by gesticulations and could repeat it after stimulation. Two patients were asked to follow simple written commands during stimulation. Both were unable to follow the command and were also unable to repeat the command after stimulation. Two patients were given simple oral commands which they could not follow during stimulation. One of the 2 patients, however, was able to remember the command and follow it after the stimulation was over.

The Token Test was also used to test for verbal comprehension. One patient was unable to follow simple one-step commands and could not remember the command after the stimulation. The other 2 patients were able to follow simple one-step commands even if it would take them more time. They also made more errors than without stimulation indicating 'I did not understand the description'. Both patients had progressively more difficulties with complex two-step commands.

Confrontation naming was one of the tests most affected during stimulation. All 3 patients were totally unable to name objects during stimulation. Interestingly, however, they were clearly aware of the object presented to them during stimulation and spontaneously, or after prompting, were able to identify during the stimulation the object by gesticulations (for example, by imitating the action of cutting with his fingers when confronted with scissors). One patient even was able whistle when confronted with a whistle. They all remembered the objects after stimulation and were able to name them correctly without any difficulty. With higher intensities of stimulation, the patients were totally unable to speak during stimulation. At lower intensities they still had major difficulties in naming the objects but not infrequently were able to define its function (for example, saying 'That is a ... clean teeth' for toothbrush). The patients had similar difficulties when asked to name objects in the room. One of them indicated that 'Words just did not enter my mind'. In 1 case at high stimulation intensities of 6 – 10 mA, the patient was totally unable to name objects in the room. At 5 mA, however, he was able to name some objects but at a considerably slower speed than without stimulation.

Repetition of simple words or sentences was tested only in 2 patients. Both patients were unable to repeat words or sentences during stimulation or to remember them after stimulation when relatively high intensities were used. At relatively lower intensities of stimulation, 1 of the patients had literal paraphasias (for example, said 'episcural' when asked to repeat 'episcopal').

Simple sequential tasks were tested only in 1 patient. Simple tasks like counting forward from 1 were performed without difficulty. However, with more difficult tasks, he had progressively more problems. For example, he was unable to count forward by 3s, list correctly the days of the week or the months of the year. On a few occasions, the patient also would switch over to a more simple sequential task that he had been asked to perform before. For example, he would start counting forwards after having started to list the days of the week. He was unaware of these errors after the stimulation was over.

All 3 patients had a severe agraphia during stimulation. Two of them were totally unable to write simple letters even when the order was given before the stimulation. One patient, however, was able to write letters and two digit numbers but had a striking agraphia for more complex tasks. One of the patients commented that he could 'not remember what to write down' during stimulation. Two patients wrote down something they had already written before stimulation even if a different order had been given this time. At lower intensity 1 of the patients was able to follow the command correctly but made spelling errors (wrote down 'limbs are quite sour' when asked to write 'limes are quite sour').

One patient, at relatively low stimulus intensities, was asked to spell a word and then to read it. He misspelled the word and then was unable to read it. Two patients were tested for copying simple words from a card. Both had no difficulty writing the word during stimulation but they were unable to read it during stimulation. After stimulation, however, they could say the word from memory.

One patient was tested for written mathematics and was unable to solve a simple problem (summation of two digits) during stimulation. He was able to remember the problem after stimulation and solve it without any difficulty. The other 2 patients were asked to solve mathematical problems given orally and were totally unable to do so even for simple addition of two digits. They could also not remember the problem after stimulation or signal during stimulation with their fingers what digits they were asked to sum.

Two patients were tested with moderately complex Koh's blocks tasks and had no impairment during stimulation. All 3 patients also were able to copy complex geometrical designs (shown during stimulation for 1 – 2 s and then asked to copy from memory also during stimulation). Finally, 1 patient was asked to imitate facial expressions after confrontation with different photographs. He had no difficulty with this task during stimulation or in remembering the facial expression after the stimulation was over.

In 4 of the 8 cases all the basal temporal electrodes which elicited language interference by stimulation also showed interictal or ictal epileptiform discharges. In 2 other cases, epileptiform discharges occurred only at some but not all basal temporal electrodes which caused language disturbances when stimulated. In 2 other cases, none of the basal temporal electrodes that produced language disturbances showed epileptiform discharges. The approximate anatomical location of the electrodes at which language disturbances were elicited by electrical stimulation was defined by the analysis of skull x-rays with the electrodes in place and corresponded to the fusiform (temporo-occipital gyrus) in all cases. In 3 cases the location of the electrodes was defined with more precision during surgery, confirming that they were overlying the fusiform gyrus. In all cases, the 'language electrodes' appeared to be located at least 3 cm posterior to the tip of the temporal lobe. In 5 cases, the exact anteroposterior extent of the language area was measured during surgery. The anterior border was consistently 3 – 3.5 cm from the anterior pole of the temporal lobe. The posterior border of the language area varied between 4 and 7 cm from the anterior temporal pole.

All 8 cases with a basal temporal speech area had an extensive resection of the medial temporal structures including the amygdala and more than 4 cm of the hippocampus. In 5 cases, the resection of the temporal lobe was tailored in such a way that no basal temporal language area was included. In 2 cases, the basal temporal language area was resected completely, and in 1 case, a limited postsurgical infarct occurred which destroyed completely the basal temporal language area. This case was operated on for a second time, and the diagnosis of an infarct of the basal temporal region was confirmed by gross inspection during surgery and pathological examination. The language test battery administered 6 months postoperatively showed no significant change in 3 cases including 2 patients in whom the basal temporal speech area was resected. One of these patients had suffered an extensive infarct of the basal temporal region including the basal temporal speech area. This patient had a mild receptive aphasia in the immediate postoperative period (only tested informally at the bedside) which had disappeared completely 6 months later during formal testing. Two of the other 4 patients who had a limited temporal resection with sparing of the basal temporal speech area showed a mild improvement in language function 6 months postoperatively; 1 had slight improvement of auditory comprehension, whereas the other showed slight improvement of auditory comprehension, verbal fluency and written expression. The other 3 patients showed mild losses. One of the patients had mild deterioration of an already preoperatively present moderate mixed aphasia. The second patient showed a slight decrease of his ready comprehension and the third patient (who had also a resection of the basal temporal language area) revealed a significant increase of literal paraphasics postoperatively.

DISCUSSION

This study confirms our previous observation based on a case report (Lüders *et al.*, 1986*a*) that the basal temporal region of the dominant hemisphere participates in the processing of language.

The possibility that the deficit produced by electrical stimulation was unrelated to any language interference (e.g., a nonspecific disturbance of consciousness or an apraxia of speech-related muscles) was excluded by careful testing. Stimulation elicited no observable muscle twitches of the tongue or perioral muscles and also no negative motor effect was observed (Lüders *et al.*, 1983, 1990). The possibility that the language interference was just an expression of a nonspecific disturbance of consciousness is also unlikely. In all cases, the language disturbance was elicited on numerous occasions without artefact, but it is impossible to exclude the possibility of epileptiform discharges occurring only during stimulation. The fact, however, that the patients were able to perform extremely complex nonverbal functions (e.g., pasting a stamp and closing an envelope, or copying a complex design during stimulation at an identical stimulus intensity) clearly points to the specificity of the language deficit. The observation that the patient was able to identify an object by gestures but was unable to name it (e.g., to imitate the act of sawing when confronted with a picture of a handsaw) also demonstrated this. The occasional occurrence of circumlocutions points in the same direction. In this context it is important to stress the fact that language disturbances occurred not infrequently at electrodes which gave no evidence of epileptogenicity (ictal or interictal). Similar selective disturbances of language were usually observed at Wernicke's area and less frequently at Broca's area. In other locations, an apparent interference with language was invariably associated with either (1) an after-discharge and inability to perform even simple nonverbal tasks (epileptic absence), (2) a negative motor effect (Lüders *et al.*, 1983, 1990), or (3) a positive motor effect. Negative motor effects were noticed most frequently in the premotor region (particularly in the inferior frontal region corresponding approximately to Broca's area) and positive motor effects occurred exclusively in the perirolandic area and in the supplementary motor region. We have examined only 6 cases with chronic subdural electrodes placed over the supplementary motor area (Dinner *et al.*, 1987). These cases showed dramatic bilateral positive motor effects, bilateral negative motor effects and sensory effects. No language-specific deficits as described by Penfield and collaborators (Penfield and Jasper, 1954; Penfield and Roberts, 1959) were observed in this small sample.

It is not surprising that previous studies of the effect of electrical stimulation of the human cortex did not describe a basal temporal language area. Most of them were performed during surgery. Due to time limitations, the surgeon usually restricted the stimulations while testing for language interference to areas in which previous evidence suggested the existence of language-related functions (mainly Wernicke's and Broca's area). There is only very limited evidence in the literature which would implicate the basal temporal region in the processing of language (Mills and Martin, 1912; Nielson, 1946; De Renzi *et al.*, 1987). Excision of the anterior 5–6 cm of the dominant lobe basal temporal region is apparently not associated with any language deficit. The only reported complication was a variable degree of verbal memory deficit (Hermann and Wyler, 1988). Additionally, the basal temporal region is not readily accessible during surgery and special electrodes would be required to stimulate the fusiform gyrus. Depth electrodes usually do not permit recording or stimulation of the basal temporal region. In our laboratory, the basal temporal language area was discovered by implanting chronic basal temporal electrodes and screening systematically all electrodes for language disturbances ('reading aloud' test).

It was interesting to notice that the basal temporal language area was located invariably in the fusiform (occipitotemporal) gyrus at the tip of the basal temporal region. In all these cases, there was no language-related function in the inferior temporal gyrus and, therefore, the basal temporal language area has to be considered as an entity separate from Wernicke's area which is usually situated in the posterior portion of the superior temporal gyrus. The white matter underlying the basal temporal language area is, however, in direct contact with the white matter underlying Wernicke's area (fig. 2). This proximity could be one factor that favours close interaction between Wernicke's area and the basal temporal language area.

Electrical stimulation of the basal temporal region produces language deficits that are almost identical quantitatively and qualitatively with the ones observed when stimulating Wernicke's area. There are, however, major differences between these areas. As mentioned above, resection of Wernicke's area is usually associated with a severe fluent aphasia, whereas there is no clear evidence for neurological deficit following resection of the anterior tip of the temporal lobe. In this context, it is interesting to note that Penfield and Roberts (1959) reported language deficits following resection of basal temporal and inferior temporal cortex. They related these deficits to the excision of the inferior temporal cortex, quoting the evidence provided by Mills and Martin (1912) and by Nielsen (1946) that the posterior-inferior temporal gyrus of the dominant hemisphere was a 'naming center' or 'language formulation area'. Heilman *et al.* (1972) observed a selective anomic aphasia in 4 out of 10 patients who had an anterior temporal lobectomy. It could be speculated that these deficits were related to lesions of the basal temporal speech area, but in our experience resection of the basal temporal speech area was not associated with any lasting speech deficit.

In this series, we were able to define Broca's and Wernicke's area significantly more frequently than the basal temporal speech area. Initially, we assumed that this was because

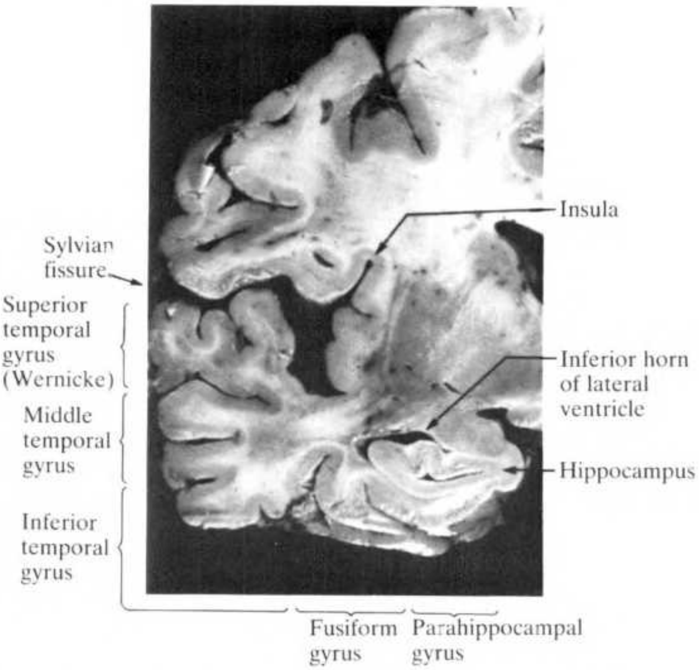

FIG. 2. Section through the temporal lobe, including the basal temporal language area in the fusiform gyrus and Wernicke's area in the superior temporal gyrus.

the basal temporal speech area is usually located more posteriorly than the location in which we implanted the subdural electrodes. However, in the cases in which we were able to locate the basal temporal region it was always located relatively more anteriorly with a clearly defined posterior margin at approximately 7 cm from the tip of the temporal lobe, an area usually covered by the 4×4 basal temporal plate. Another possibility was that the basal temporal speech area was of a relatively smaller size and, therefore, could be 'missed' more frequently. A comparison of the number of electrodes which cover the basal temporal speech area with the number of electrodes which cover Broca's area does not support this hypothesis either. Both areas tend to be covered by approximately the same number of electrodes. We also considered the possibility that the basal temporal speech area could represent a pathological Wernicke's area 'displaced' to the basal temporal region because of pathology affecting Wernicke's area. In the majority of these patients, however, the main epileptogenic focus was in the basal temporal region (not in the temporal convexity) and in 6 of the 8 patients with a basal temporal speech area, an independent well-defined Wernicke's area was also identified by electrical stimulation on the lateral convexity of the temporal lobe in the vicinity of the sylvian fissure, and clearly differentiated from the basal temporal language area. Finally, we could assume that in some cases the basal temporal speech area is dispensable because of compensatory mechanisms. This would be similar to the anatomical substrate sustaining memory which most probably has a bilateral distribution and, therefore, memory deficits only become clinically evident when damaging both medial temporal regions (Milner, 1967). The 8 cases in which we elicited major language disturbances with stimulation of the basal temporal region would be cases in which access to compensatory mechanisms would be significantly delayed. The fact that some of these cases had no language disturbances after removal of the basal temporal language area seems to indicate that with time, even these cases have access to effective compensatory mechanisms.

In the first few cases in which electrical stimulation demonstrated a basal temporal language area, we carefully tailored the final temporal resection to avoid a lesion of the basal temporal language area. In 1 case, however, an infarct of the basal temporal region occurred as a complication of the surgical procedure. This case had a temporary slight aphasia in the immediate postoperative period but recovered completely when tested formally 6 months postoperatively. After that experience, we decided to resect all the epileptogenic tissue in the basal temporal region even if the results of electrical stimulation had demonstrated a basal temporal speech area. So far, we have resected the basal temporal language area in 2 cases with no language deficit exceeding the deficits observed in the earlier cases in which the resection did not include the basal temporal language area. The material, however, is still too small to analyse quantitatively the effect of resection of the basal temporal area or language functions. In 1 case a significant drop in verbal memory was seen. In all these cases, however, a significant resection of the hippocampus was also performed, and therefore it is quite likely that the drop of memory function is due to the lesion of the hippocampus. Similar drops of verbal memory function were also detected after excision of the dominant hippocampus with careful sparing of the basal temporal language area and in cases in which electrical stimulation of the basal temporal electrodes had not demonstrated language deficits.

The language deficit produced by stimulation of the basal temporal region was striking, and essentially can be categorized as a global aphasia. At high intensities of stimulation there was total inability to comprehend written or spoken language, with a striking alexia, agraphia, acalculia and complete anomia and inability to repeat even simple words. This striking deficit was in clear contrast with the nonverbal performance during electrical stimulation which was essentially intact. The patients had no problems with Kohs' Blocks, were able to copy complex geometrical designs and to imitate faces without difficulties. They were even able to copy single letters or simple words (in spite of a striking agraphia) which they apparently handled like complex designs as opposed to symbols of a defined verbal meaning. The clearest dissociation between verbal and nonverbal tasks was apparent when these patients were asked to name objects by confrontation. They were able to identify objects by gesticulation but were totally unable to name them.

From the highly selective language deficits described above, it is tempting to speculate that during stimulation these patients suffered a total inability to transform sounds or images with verbal meaning (letters, words, sentences or numbers) in understandable concepts. Additionally, the opposite function of generating verbal symbols (e.g., words) in response to nonverbal stimuli was also grossly affected. This suggests that the patients had no access to the verbal engrams which establish the link between symbolic verbal material and the corresponding nonverbal expressions. In other words, these considerations lead us to consider that the basal temporal language area could correspond to what Wernicke called the 'Wortschatz' which he assumed was most probably located in the dominant temporal lobe close to the posterior language area. Our findings suggest that the 'Wortschatz' area is not located around the posterior language area but actually coincides with the basal temporal language area.

It was also interesting to note in these patients that a change in the intensity of stimulation significantly modified the type of aphasia. With relatively low levels of stimulation, the patients were able to perform some verbal functions, and this gave us an opportunity to evaluate which of the different verbal deficits were affected most severely. For example, at low stimulation intensities, the patients were able to read, even if relatively slowly, and could repeat simple words but with literal paraphasias. They could also perform sequential tasks, but with more complex sequences there was a tendency to perseverate (they returned to the simpler sequence during the stimulation period). They were even able to spell some simple words but with frequent misspellings. In clear contrast with the improvement in the functions enumerated above, lower stimulus intensities were consistently associated with a more or less complete anomia even for very simple words. This last observation would be consistent with the hypothesis that these patients have essentially no access to verbal engrams as was explained above.

These studies also point out very clearly the striking differences between the results obtained from lesions (e.g., strokes or tumours) and from studies of electrical stimulation. Well-documented lesions during surgery in 3 of the cases reported here were not associated with any language deficit postoperatively. These observations suggest that most probably in patients with permanent lesions of the basal temporal language area compensatory mechanisms become effective. It is possible that verbal engrams are stored, not only in the basal temporal language area, but also at other sites. During an abrupt interference of the basal temporal language area, as produced by electrical stimulation, the patient would not have sufficient time to gain access to engrams stored elsewhere. With time, however, the patient could access effectively intact engrams stored elsewhere. This same phenomenon could also explain why with prolonged stimulation the patients have a tendency to recover some functions at the end of the stimulation. Another interesting difference with lesion studies is the fact that inactivation of a very small cortical area by electrical stimulation is frequently sufficient to produce a striking global aphasia. This is in clear contrast with lesions which usually only produce aphasias with relatively extensive dominant lobe cortical lesions. This difference could also be related to the acuteness of the electrical stimulation which does not allow for compensatory mechanisms.

Finally, it is interesting to note that electrical stimulation produces relatively similar deficits in the 3 language areas we were able to investigate systematically: Broca's, Wernicke's and the basal temporal language areas. In all these areas, electrical stimulation elicited speech arrest and clear comprehension deficits (Lesser *et al.*, 1986; Lüders *et al.*, 1986*a, b*). The only important difference was the observation that in Broca's area electrical stimulation frequently also produced a negative motor effect which affected preferentially voluntary muscles involved in fine movements including those participating in speech (Lüders *et al.*, 1983, 1991). From these observations the question arises as to whether the difference between the predominantly expressive aphasias with lesions in Broca's area are due to the simultaneous involvement of language and negative motor areas, whereas lesions of Wernicke's area, where no negative motor area exists, are always limited to a selective involvement of language areas. In other words, the hypothesis which evolves from these stimulation studies is that lesions of selective language circuitry involving any of these 3 language centres would be relatively uniform. Interference at any point within this probably closely interconnected system produces almost identical deficits. The differences noted in clinical lesions studies would not be the results of selective damage to language centres but the result of an associated deficit secondary to lesions of independent systems anatomically closely associated with language areas. A typical example would be a lesion of Broca's area which, according to this hypothesis, would be usually associated with a marked expressive aphasia because of the anatomical proximity of the negative motor area.

From the discussion presented above emerges the hypothesis that Wernicke's language area functions in close association with the basal temporal language area. Identification of the symbolic meaning of auditory or verbal words, which classically is considered a function of Wernicke's area, is only achieved by activation of verbal engrams which we assume are stored in the basal language area. Like other memory functions, the engrams would be stored bilaterally but access to the dominant basal temporal language area is relatively faster because that pathway is used on a routine basis. The assumption of a close interaction of the basal temporal and Wernicke's language area would require extensive direct anatomical connections between these two language areas. Indeed, studies by Rosene and Van Hoesen (1977) demonstrated that in monkeys the parahippocampus acts as a relay station of the extensive connections that exist between the hippocampus and the neocortex. These anatomical connections are certainly consistent with the hypothesis that Wernicke's and the basal temporal language areas interact extensively in the processing of verbal material. A diagrammatic representation of the way this interaction can be conceived has been presented elsewhere (Lüders *et al.*, 1987).

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(Received August 23, 1988. Revised November 3, 1989. Accepted April 24, 1990)

Brain (1991), **114**, 755–759

ILLUSIONS OF HEAD AND VISUAL TARGET DISPLACEMENT INDUCED BY VIBRATION OF NECK MUSCLES

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SUMMARY

Vibration of the posterior muscles of the neck in human subjects induces illusions of displacement and movement of a visual target when there is no visual reference (Biguer *et al.*, 1988). Although illusions of head movement are rarely reported by subjects, when they point to the location of the nose they demonstrate an alteration of the perceived position of the head. The kinaesthetic illusion is in a direction consistent with the visual illusion but is of smaller magnitude.

INTRODUCTION

The vibration of muscles or muscle tendons alters proprioceptive input and produces kinaesthetic illusions in human subjects. Goodwin *et al.* (1972) showed that the vibration of the biceps tendon evokes an illusion of extension of the elbow. This illusion involves both an altered perception of the position of the arm and a sense of continuing movement (McCloskey, 1973). Muscle spindle afferents are sensitive to percutaneous vibration (Burke *et al.*, 1976; Roll and Vedel, 1982) and the illusions produced by vibration of limb muscles (Goodwin *et al.*, 1972; Lackner and Levine, 1979) can be accounted for as the interpretation of this vibration-induced discharge as lengthening of the vibrated muscles.

Recently, vibration of the posterior muscles of the neck has been shown to induce illusions of changed position and of motion of a visual target when the target is presented with no visual background (Biguer *et al.*, 1988). The illusions are consistent with altered proprioceptive input from the neck being used to judge the direction of gaze. However, few subjects report any illusion of movement of the head relative to the body and Biguer *et al.* proposed that 'the influence of proprioception can be restricted to the system that defines visual direction and motion while leaving unaltered the perception of head posture on torso'. In the present study we confirmed the presence of a visual illusion induced by vibration of the neck muscles and used an objective measure of perceived head position to identify an associated illusory alteration of head posture.

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METHODS

Thirteen subjects, who were students and staff of the University of New South Wales, took part in the experiments. Informed consent, according to the guidelines of the institution, formulated in accordance with the Declaration of Helsinki, was obtained from all subjects. Four were discarded from the study because no consistent illusion of movement of the visual target could be achieved.

Subjects sat with the head fixed in position by a bite bar (fig. 1). A physiotherapy vibrator was mounted behind the subject so that vibration (100 Hz) could be applied below the left occiput just lateral to the spine. Subjects rested the face against a moulded eyepiece and looked binocularly into an enclosed tube at the end of which was a small red light-emitting diode (LED). All external light was excluded so that there was no visual reference apart from the target LED. Beyond the tube, behind the visual target, at approximately 25 cm from the subject was a board with a grid marked on the side facing away from the subject. The apparent position of the visual target could be indicated by the subject bringing the index finger from behind the LED and placing its tip on the grid at the appropriate position. A second grid, fixed below the eyepiece, masked the face. It was positioned so that it touched the tip of the subject's nose. The apparent position of the tip of the nose could be indicated by the subject placing the fingertip on this grid at an appropriate position. This gave an objective measure of the perceived position of the head.

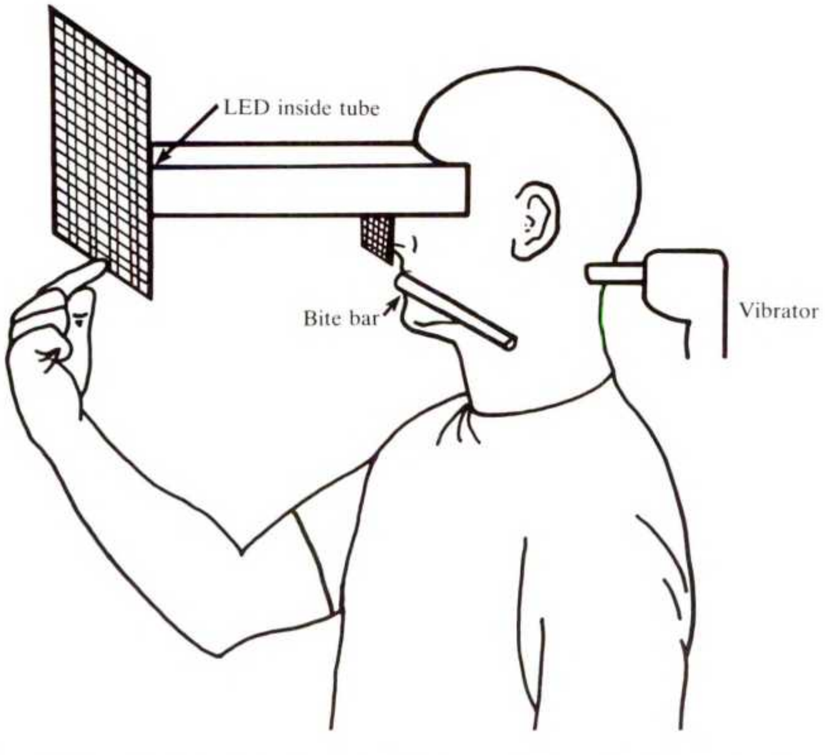

FIG. 1. The subject was seated with the head secured in position by a bite bar. A physiotherapy vibrator was applied to the neck. The subject looked at a small LED inside a tube from which all other light was excluded, and indicated the apparent position of the LED on a grid. The perceived position of the nose could be indicated on a second grid close to the face.

At the start of an experiment the position of the tip of the physiotherapy vibrator was adjusted until subjects indicated that the visual target appeared to move and that the movement had a large horizontal component (*cf* Biguer *et al.*, 1988). Very small changes in the position of the vibrator could cause large alterations in the magnitude of the illusory movement of the light and could even reverse its direction. When the vibrator was appropriately positioned, subjects performed twenty trials. Each trial consisted of (1) pointing to the visual target by touching the grid with the right index finger (a mark on the fingernail allowed the grid reference to be read off and recorded) and returning the arm to a resting position; (2) after 15 s of vibration of the neck and while vibration continued, again indicating the position of the LED; (3) touching the grid over the tip of the nose to indicate its felt position while vibration continued; and (4) 15 s after the cessation of vibration repeating the indication of the position of the tip of the nose. Thus for each trial the apparent movement of the light and of the nose could be calculated. We found it important to measure the perceived positions both of the target LED and the head in the same period of vibration because the sensitivity of the perceptual illusions to the positioning of the vibrator made successive trials of vibration slightly different from each other. To allow direct comparison both perceived movements were expressed as angles of apparent head rotation.

RESULTS

Nine subjects experienced a consistent visual illusion. For 3 the target appeared to move to the left and for the other 6 the target light moved to the right. For each of these subjects the mean apparent displacement of the light was statistically significant ($P < 0.001$). Fig. 2 (open columns) shows the magnitude of the mean illusory displacement expressed as degrees of head movement for each subject. This ranges between 3.3° and 7.5°. It also shows each subject's mean perceived change in head

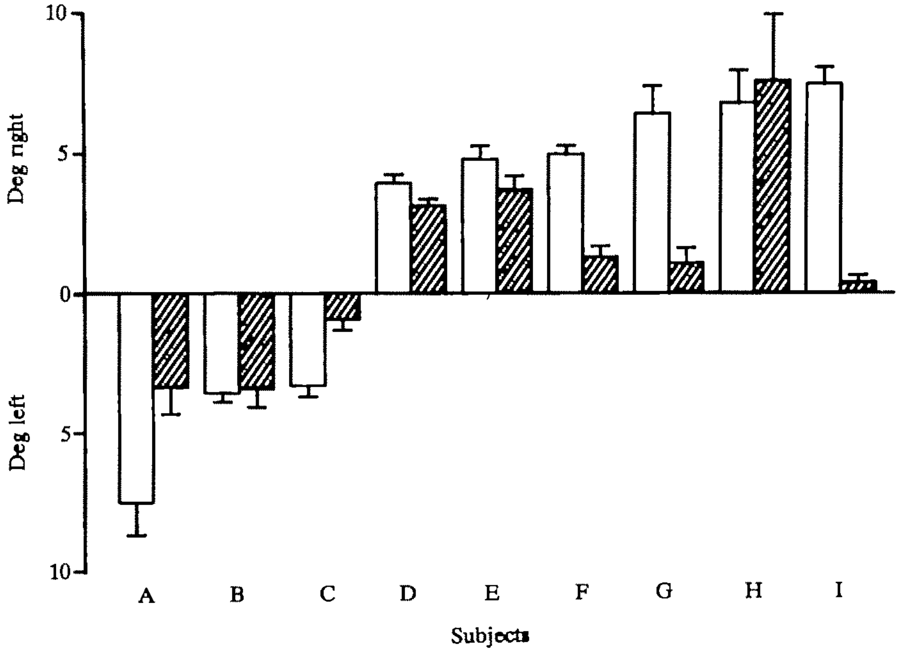

FIG. 2. The perceived angular displacement of the LED during vibration of the neck is represented by the open bars. The mean and SEM for each subject are shown. For 3 subjects, the light appeared to move to the left and for the other 6, to the right. The shaded bars show the mean perceived angular displacement of the head (indicated by pointing to the nose).

position (shaded columns). This was not significant for every subject but over the group of subjects the illusory displacement of the head in the same direction as the visual illusion was significant ($P < 0.01$). Correlation of the kinaesthetic and visual illusions gave a coefficient of 0.76 ($P < 0.02$). However, a paired t test showed that apparent head displacement was significantly less than visual ($P < 0.02$).

Although all subjects reported seeing the light move, only 1 subject reported a sensation of the head turning on the trunk. On questioning, other subjects reported some disorientation and dizziness associated with the vibration but no sensation of head movement or displacement.

DISCUSSION

The study confirms that an illusion of displacement of a visual target can be elicited by vibration of the posterior muscles of the neck. Subjects also reported the illusion of continuing movement of the light but no objective measures of this perception were made here. As in the study of Biguer *et al.* (1988), the anatomy of the neck muscles makes it difficult to be sure of which muscle or muscles were vibrated. Apparent movement of the target light to the right is consistent with vibration of the left splenius capitis which rotates the head to the left while vibration of the sternomastoid, the insertion of which is in close proximity to splenius capitis would be expected to cause an illusion of movement in the opposite direction as it turns the head to the right. An object with fixed retinal position would be interpreted as an object moving in the direction of head rotation. Subjects also reported up and down components to the visual illusions but these vertical movements of the target light were minimized here by careful positioning of the vibrator head and were not analysed.

Alteration of proprioceptive input from the neck by vibration led to a changed perception of head position as well as the illusion of displacement of the visual target. The kinaesthetic and visual illusions were correlated but the kinaesthetic illusion was smaller and varied more between subjects.

Signals from the vibrated muscles were not the only available input that could contribute to subjects' perception of head position. Proprioceptive signals from the unvibrated muscles of the neck, cutaneous input from the face against the eyepiece and pressure of the bite bar on the teeth all signalled that the head had not changed position. These inputs contradicted the signals of muscle lengthening from the vibrated muscle and may have overshadowed them in the way vision of a vibrated limb inhibits the illusion of limb movement (Lackner and Taublieb, 1984) and the presence of a visual background counteracts the illusion of movement of the visual target (Biguer *et al.*, 1988). Intersubject differences in the expression of the illusion of head displacement and of the visual illusion may well depend on the relative attention given to these inputs by each subject.

In addition to proprioceptive and somatosensory inputs, the vestibular apparatus is sensitive to movement of the head in space. However, the lack of vestibular input is probably not influential here. The angular velocity of the movement component of the visual illusion is slow (< 0.2 deg/s; Biguer *et al.*, 1988) and a matching head movement of this velocity would be below the detection threshold of the vestibular apparatus (Benson *et al.*, 1989).

The presence of a kinaesthetic as well as a visual illusion indicates that proprioceptive information from the neck is used both for determination of head position and for determination of the direction of gaze. It is possible that this proprioceptive information, along with relevant cutaneous and vestibular signals, could be processed to give a signal of the position of the head on the torso, and that this derived head position then could be combined with an eye-in-head signal to determine the direction of gaze. However, if this occurred, the magnitude of the visual illusion would be expected to be the same or smaller than the illusion of head rotation. The manipulated proprioceptive input would be incorporated in the head position signal and any other input contributing to gaze direction could only diminish the visual illusion by signalling a lack of movement.

In this study, the illusion of displacement of the visual target was of greater magnitude than the illusion of head rotation and this suggests that determinations of the direction of gaze and position of the head on torso use the proprioceptive inputs from the neck independently. That is, the proprioceptive signal from the neck is not first interpreted, along with other relevant sensory input, as a head-on-trunk signal and then combined with an eye-in-head signal to derive the direction of gaze, but direction of gaze and position of the head are processed in parallel so that here inputs that acted to negate the illusion of head turning did not affect the apparent direction of gaze.

ACKNOWLEDGEMENT

This study was supported by the National Health and Medical Research Council of Australia.

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(Received February 28, 1990. Accepted April 26, 1990)

Brain (1991), **114**, 761–774

PERIVENTRICULAR LESIONS IN THE WHITE MATTER ON MAGNETIC RESONANCE IMAGING IN THE ELDERLY

A MORPHOMETRIC CORRELATION WITH ARTERIOLOSCLEROSIS AND DILATED PERIVASCULAR SPACES

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SUMMARY

Magnetic resonance imaging (MRI) was performed postmortem on the brains of 40 patients aged over 60 yrs who had died from causes other than brain disease. Periventricular lesions of increased signal intensity on T_2-weighted images, graded as moderate or severe, were found in 10% of the patients in the age group between 60 and 69 yrs, and in 50% between 80 and 89 yrs. Macroscopic and microscopic whole-brain sections were studied in 19 brain specimens (8 with normal white matter, 4 with moderate lesions and 7 with severe lesions of the white matter on MRI). The presence or absence of periventricular lesions on MRI correlated well with the severity of demyelination and astrocytic gliosis. Demyelination was always associated with an increased ratio between wall thickness and external diameter of arterioles (up to 150 μm). A variable degree of axonal loss in Bodian-stained sections was present in the white matter of all brains with demyelination. Dilated perivascular spaces were found and studied morphometrically in 9 brain specimens; their presence correlated strongly with corrected brain weight, but incompletely with demyelination and arteriolosclerosis. Our findings suggest that arteriolosclerosis is the primary factor in the pathogenesis of diffuse white matter lesions in the elderly. This is soon followed by demyelination and loss of axons, and only later by dilatation of perivascular spaces.

INTRODUCTION

The improvement of imaging techniques of the brain in the last decade has drawn attention to the occurrence of diffuse lesions of the white matter, usually termed periventricular leukoencephalopathy (Valentine *et al.*, 1980; Goto *et al.*, 1981; Loizou *et al.*, 1981). Patients with symmetrically diminished density of the white matter on computed tomography (CT) were often found to have some degree of cognitive impairment or even manifest dementia, or focal neurological signs (Steingart *et al.*, 1987*a, b*; Gupta *et al.*, 1988). Magnetic resonance imaging (MRI) has proved more sensitive than CT in detecting these white matter lesions (George *et al.*, 1986; Erkinjuntti *et al.*, 1987). The hypodense lesions on CT correspond with areas of increased signal intensity in T_2-weighted MR images. Periventricular and focal lesions on MRI have been

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recognized particularly in patients with stroke (Awad *et al.*, 1986*a*; Gerard and Weisberg, 1986), in patients with vascular dementia (Hershey *et al.*, 1987), and in subjects with risk factors for stroke (Awad *et al.*, 1986*a*; Gerard and Weisberg, 1986; Lechner *et al.*, 1988). The extent of the periventricular lesions may range from narrow rims to involvement of the entire white matter, with the exception of the subcortical U fibres. Focal lesions in the deep white matter may be single, multiple or confluent.

Microscopic study of brains of patients with leukoencephalopathy on CT showed diffuse demyelination of the white matter, multiple infarcts in the basal ganglia and arteriolosclerosis (Caplan and Schoene, 1978; Rosenberg *et al.*, 1979; De Reuck *et al.*, 1980). These findings correspond with the classical description of subcortical arteriosclerotic encephalopathy or Binswanger's disease (Binswanger, 1894; Olszewski, 1962). On neuropathological study of single or few cases investigated by MRI, large periventricular lesions of increased signal intensity in T_2-weighted images corresponded with extensive demyelination, cystic lacunes, and arteriolosclerosis (Kinkel *et al.*, 1985; DeWitt *et al.*, 1987; Révész *et al.*, 1989). In postmortem MRI studies of a series of brains with slight lesions of the white matter, dilatation of perivascular spaces was proposed as the microscopic correlate (Awad *et al.*, 1986*b*; Kirkpatrick and Hayman, 1987), whereas others found true infarcts with necrosis, axon loss and gliosis (Braffman *et al.*, 1988; Marshall *et al.*, 1988). Both dilatation of perivascular spaces and lacunar infarction often occur in association with hypertension, and are frequently seen in the same brain specimens. In the neuropathological literature about Binswanger's disease, the focal abnormalities accompanying the diffuse loss of myelin have mostly been described as cystic infarcts (Olszewski, 1962; Caplan and Schoene, 1978; De Reuck *et al.*, 1980), and only a few studies mentioned dilatation of perivascular spaces (Garcin *et al.*, 1960). Only recently renewed attention has been paid to the distinction between these two abnormalities (Poirier and Derouesné, 1985; Benhaïem-Sigaux *et al.*, 1987).

In the present study of a series of 40 brains of patients over 60 years of age, we tried to elucidate the pathological correlates of white matter lesions on MRI, with actual measurement of arteriolar thickening and of the number and size of dilated perivascular spaces.

MATERIAL AND METHODS

Patients

MRI examination was performed on the brains of 40 consecutive patients over the age of 60 yrs who came to autopsy. We excluded only patients in whom brain disease contributed directly to death or in whom brain surgery had been performed. Patients with dementia or epilepsy were not excluded. The cause of death was cardiovascular disease in 23 patients, infectious disease in 9, cancer in 6, and miscellaneous in 2 patients. The mean age of the patients was 76.2 (SD ± 8.1) yrs; 19 were female and 21 male. Thirteen patients had been cared for in psychogeriatric hospitals. One patient had been treated by ventriculoperitoneal shunting for suspected normal pressure hydrocephalus, but without subsequent improvement.

MRI technique and assessment

The brains had been fixed in 4% formaldehyde solution for 1–68 days. They were examined in a cylindrical container filled with formalin, with the convexity of the hemispheres lying on the bottom of the container. The brain was orientated in such a way that the optic chiasm and brainstem were exactly in the sagittal scanning plane. MRI was performed with a 1.5 T Philips gyroscan. First, a sagittal scanogram was produced. Then images were acquired in the axial plane with a slice thickness of 7 mm and a slice increment of 1.4 mm in all brains. Multiple slice spin-echo (SE) sequences were performed with a repetition time (TR) of 2000 ms and an echo time (TE) of 50 and 100 ms, producing a T_2-weighted image. Identically orientated slices in the axial plane were acquired with an inversion recovery (IR) sequence, in 37 of the 40 brains. A TR of 1500 ms, inversion time (TI) of 575 ms and a TE of 30 ms were used, producing a heavily T_1-weighted image. In this fashion, exact comparison of T_1 and T_2-weighted images was possible. Coronal MRI slices of severe lesions were made in a few cases to localize these lesions more accurately.

Two investigators judged the MRI scans for the presence and degree of periventricular lesions in the white matter, as evident from increased signal intensity in T_2-weighted images or a low signal-intensity in the heavily T_1-weighted images. The MRI scans were classified in 3 groups: normal white matter, moderate periventricular lesions or severe periventricular lesions. A hyperintense rim around the tip of the frontal horns was present in all brains, and was not considered abnormal. Moderate periventricular lesions involved only part of the anterior or posterior white matter. Lesions were regarded as severe if these extended from the frontal or occipital horns to the corticomedullary junction, involving all or most of the anterior or posterior white matter. In addition, the signal intensities of white and grey matter were compared in the T_1-weighted images, and were correlated with the duration of formalin fixation.

Macroscopic observations and light microscopy

Two of the 40 brains were excluded from further analysis. In 1 case with a history of trauma there were lesions in the frontobasal white matter on MRI which corresponded with areas of myelin loss on microscopic study. In 1 other case, with severe white matter lesions on MRI, there was diffuse involvement of the white matter by a non-Hodgkin lymphoma.

From the remaining 38 brains, we selected 19 brain specimens for further neuropathological and quantitative investigation. All specimens with severe periventricular lesions were selected. Brain specimens with normal white matter or with moderate lesions were selected to correspond with the age of the patients with severe periventricular lesions. Consecutive whole-brain, paraffin-embedded slices of 15 μm thickness were cut through the anterior part of the frontal horns and through the occipital horns, and were stained with haematoxylin-eosin, with Weil's stain and Luxol fast blue for myelin, with phosphotungstic acid haematoxylin (PTAH) for gliosis, with periodic acid-Schiff (PAS) for senile plaques, with elastica van Gieson for arteriolosclerosis, and with Congo red for amyloid. White matter specimens of 1 cm × 1 cm were taken from the region anterolateral to the frontal horns, up to the subcortical region, and were stained for axons with Bodian.

The white matter on Weil-stained sections was assessed by 2 experts in neuropathology, independently of each other and without knowledge of the MRI findings. Three groups were distinguished: normal white matter (group A), moderate demyelination (group B), and severe demyelination (group C). Demyelination in frontal or posterior coronal sections was judged as moderate if the pallor on Weil staining involved only part of the white matter, and as severe if the pallor extended as far as to the subcortical U fibres.

An enlarged perivascular space is defined as a round regular cavity, the wall consisting of gliosis, and with a patent arteriole Such spaces can be reliably distinguished from an artefactual space, caused by postmortem retraction of brain parenchyma from the adventitia of the arteriole. A lacunar infarct is recognized as an irregular cavity with dense astrocytic gliosis in the wall; it is caused by occlusion of a small penetrating artery (Fisher, 1965; Kappelle and van Gijn, 1986). Senile plaques in the cerebral cortex were counted in 30 low power microscopic fields taken from the frontal, temporal and parietal lobes. Amyloid angiopathy was rated as absent or present.

Quantitative analysis of arteriolosclerosis

Arteriolosclerosis consists in proliferation of the intima, degeneration of the internal elastic lamina, and hypertrophy with hyalinization of the tunica media, together resulting in thickening of the wall and narrowing of the lumen (Woollam and Millen, 1968). Camera lucida drawings at magnification ×40 were made of samples of arterioles, up to 150 μm, in the white matter of frontal and parietal sections, after elastica van Gieson staining (mean sample 45 arterioles, range 11 – 107 arterioles). After having coded the samples to avoid bias, we measured from these drawings the diameter of the cross-sectioned arterioles and their wall thickness, by means of an IBM-PC with graphic tablet. In case of obliquely sectioned arterioles, the smallest diameter and wall thickness were measured. For each brain specimen, the mean ratio between the thickness of the wall and the total diameter of the arteriole was used as a measure for the severity of arteriolosclerosis. This ratio cannot exceed the value of 0.5, in which case the lumen is occluded. The accuracy of the sampling method was determined by measuring all visible arterioles in frontal and parieto-occipital sections of 2 brains (80 and 96 arterioles) and by subsequently comparing the results with those of previous samples from the same specimen (63 and 70 arterioles, respectively). No marked differences were found between the mean ratios (0.23 vs 0.22, and 0.27 vs 0.25). Dilatation of perivascular spaces (DPS) was measured in the same manner: the calculated areas of all DPS were added together and expressed in mm^2 per cm^2 white matter.

RESULTS

Frequency of MRI lesions in relation to clinical features

Of the 38 MRI scans, the periventricular white matter had a normal signal intensity of T_2-weighted images (fig. 1A) in 25 brain specimens (mean age 73.8 yrs ± 7.2 SD).

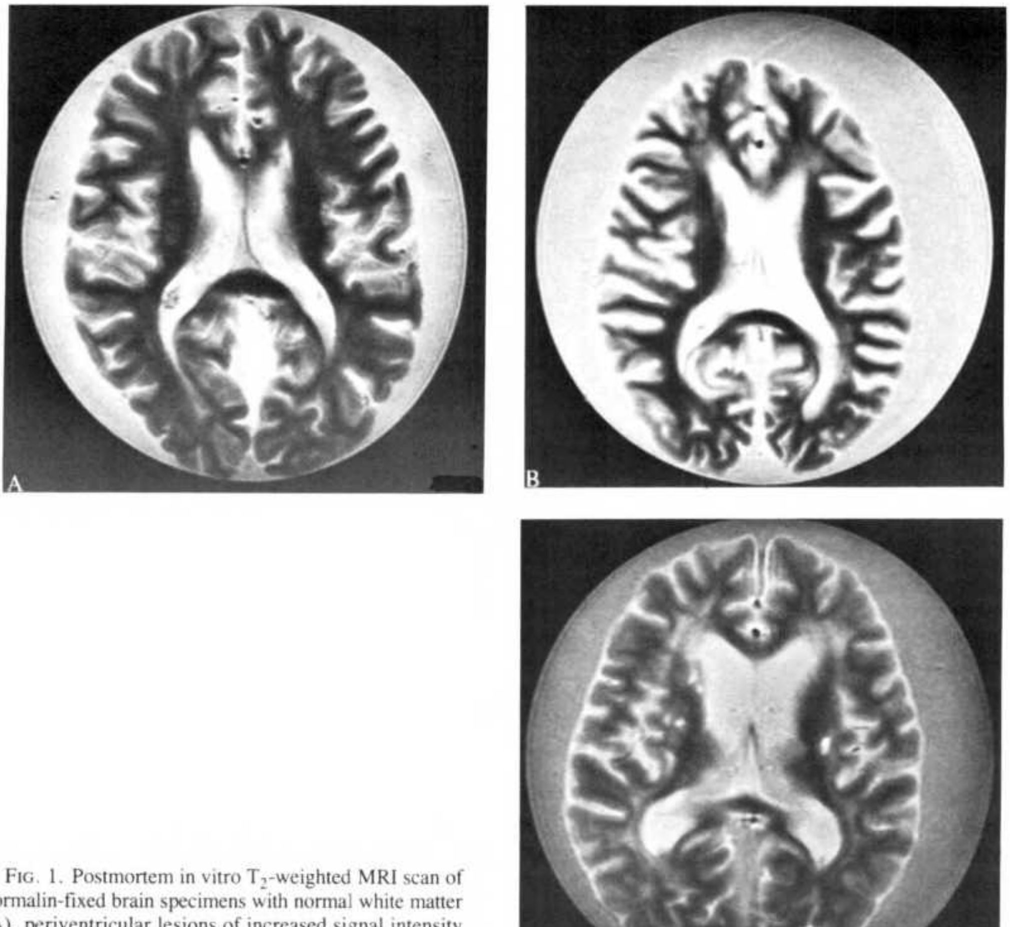

FIG. 1. Postmortem in vitro T_2-weighted MRI scan of formalin-fixed brain specimens with normal white matter (A), periventricular lesions of increased signal intensity involving only part of the white matter (B), and severe periventricular lesions extending to the subcortical region (C) (TR = 2 s, TE = 50×10^{-3} s).

Moderate lesions around the frontal and/or occipital horns (fig. 1B) were present in 6 cases (mean age 80.0 yrs ± 6.6 SD), and severe periventricular lesions (fig. 1C) were seen in 7 brain specimens (mean age 82.4 yrs ± 8.5 SD). Focal lesions in the basal ganglia and deep white matter or in the cortex were found equally often in these three groups. A reversal of grey and white matter signal intensities occurred on T_1-weighted images between 24 and 48 h of formalin fixation. A good correlation was found for the severity of lesions between the axial and the coronal plane.

The frequency of white matter lesions in different age groups is shown in Table 1. In the group aged between 60 and 69 yrs, normal white matter was found in 9 of 10 patients (90%), whereas only 6 of 12 patients (50%) aged between 80 and 89 yrs showed normal white matter on MRI. Dementia had been diagnosed in 3 of the 25 patients with normal white matter, 1 of the 6 patients with moderate lesions, and 5 of the 7 patients with severe lesions. A history of hypertension was equally frequent in the three groups.

TABLE 1 FREQUENCY OF PERIVENTRICULAR LESIONS WITH INCREASED SIGNAL INTENSITY IN T_2-WEIGHTED MR IMAGES IN DIFFERENT AGE GROUPS

Age group (yrs)	*No*	*Moderate lesions*	*Severe lesions*	*Percentage with lesions*
60–69	10	0	1	10
70–79	13	3	1	31
80–89	12	2	4	50
>90	3	1	1	67
Total	38	6	7	34

Macroscopic appearance

Of the 19 brain specimens selected for further neuropathological investigation, 9 had normal white matter at MR imaging, 3 had moderate lesions, and all 7 brains with severe periventricular lesions were included (*see* Methods). All necrotic lesions that were found on macroscopic study of one or both hemispheres had already been identified by MR imaging. Also, 2 lacunar infarcts in the pons had been well visualized by MRI. Three groups were distinguished according to the staining for myelin. Group A were 8 cases with white matter of normal appearance (fig. 2A); group B were 4 brains with moderate demyelination (fig. 2B); group C were 7 cases with severe demyelination on one or both sections, in the frontal and in the posterior region (fig. 2C). The extent of periventricular lesions on MRI correlated well with the severity of demyelination on Weil sections. Demyelination on Weil-stained sections was graded as 1 deg more severe than MRI lesions in 2 brains, and 1 deg less severe than MRI lesions in 1 brain.

Microscopic findings

A small zone of demyelination with astrocytic gliosis adjacent to the ventricle was found in all brains. This zone corresponded to the rim of high signal intensity around the ventricle on the MRI scans. Moderate or severe demyelination on Weil sections was microscopically diffuse and sometimes patchy, with astrocytic gliosis. In all brains with severe demyelination, axonal swelling and axonal loss was present in variable degree

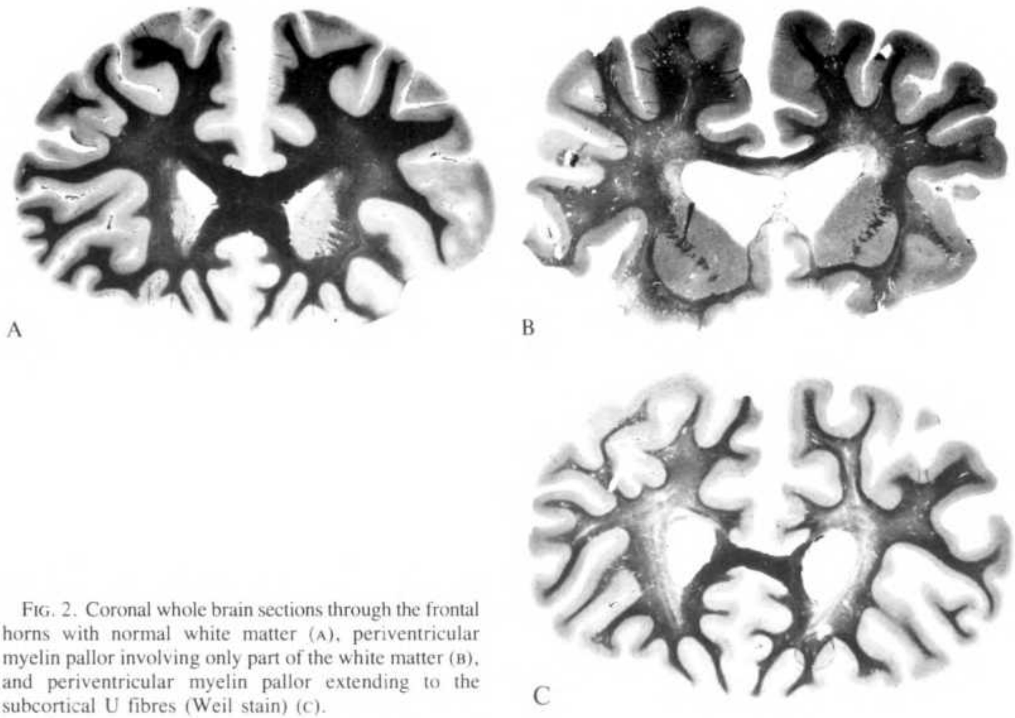

FIG. 2. Coronal whole brain sections through the frontal horns with normal white matter (A), periventricular myelin pallor involving only part of the white matter (B), and periventricular myelin pallor extending to the subcortical U fibres (Weil stain) (C).

in Bodian-stained sections. A lacunar infarct (fig. 3A) or a cortical infarct was found in 3 of the 6 brains with severe demyelination, and 5 of the 7 brains with normal white matter. Senile plaques, more than 1 per microscopic field, were found in 8 brain specimens, 3 of which also had severe demyelination of the white matter. Amyloid angiopathy, found in 5 cases, was strongly associated with senile plaques. There was no relation between the number of senile plaques and amyloid angiopathy and the severity of demyelination (Table 2). Of the 5 brains of patients with severe demyelination and dementia, 4 showed no cortical infarcts, and 2 of these showed no or only a few senile plaques.

Quantitative analysis

We measured the wall thickness of nearly 1000 arterioles (less than 150 μm in diameter) in the white matter in the frontal and parietal sections (fig. 3B). The mean diameter of the arterioles in group A was 45.2 μm $\pm$ 3.8 SD, in group B 49.8 μm $\pm$ 6.0 SD, and in group C 55.5 μm $\pm$ 3.5 SD. All 8 brain specimens with normal white matter had mean arteriolar thickness ratios below 0.20 and all 11 specimens with moderate or severe demyelination had ratios above 0.20 (Table 2, fig. 4). The difference in ratios between the three groups, calculated by means of the Kruskall-Wallis test was statistically significant ($P < 0.01$). This difference is mainly due to a difference between groups A and B.

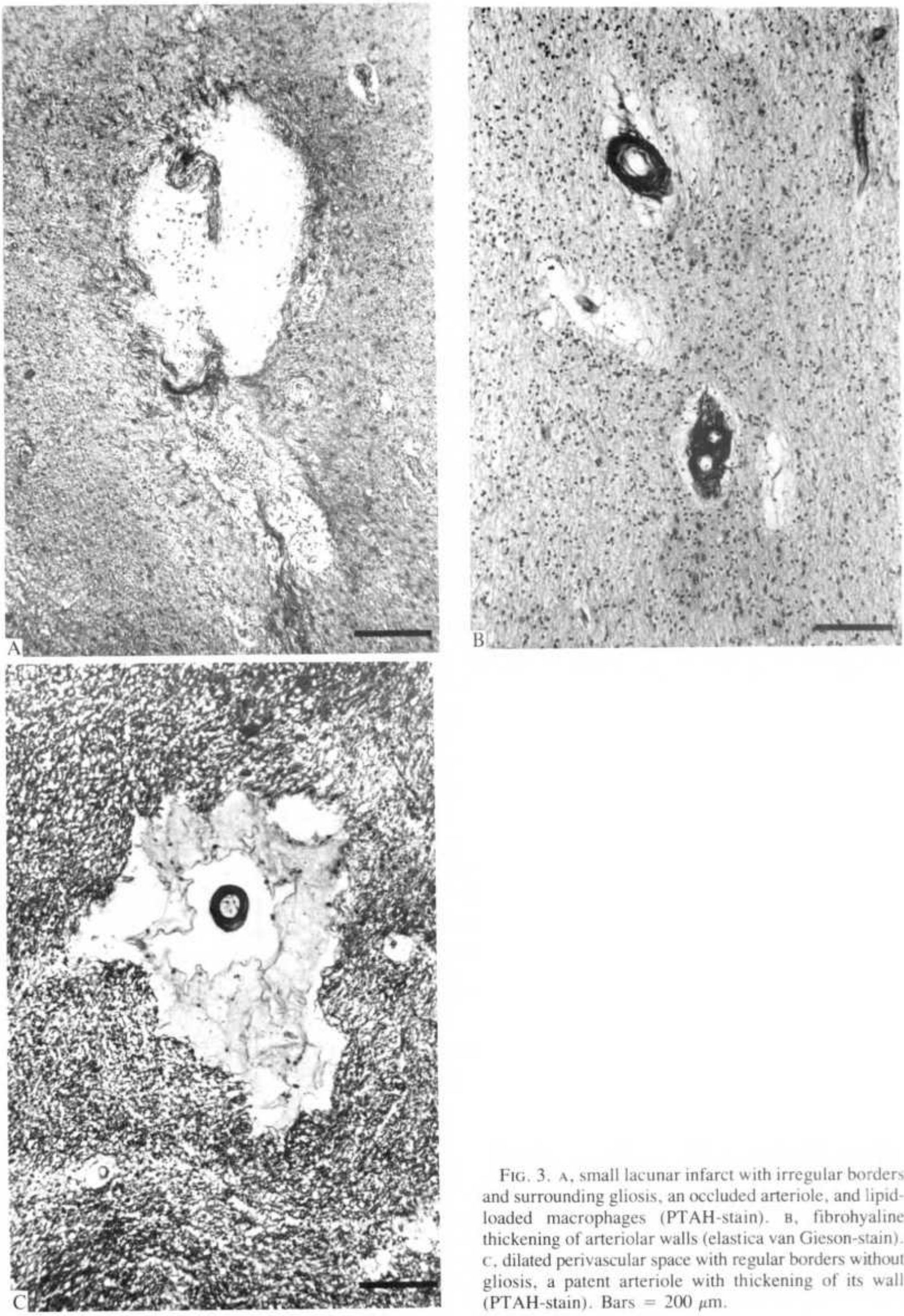

FIG. 3. A, small lacunar infarct with irregular borders and surrounding gliosis, an occluded arteriole, and lipid-loaded macrophages (PTAH-stain). B, fibrohyaline thickening of arteriolar walls (elastica van Gieson-stain). C, dilated perivascular space with regular borders without gliosis, a patent arteriole with thickening of its wall (PTAH-stain). Bars = 200 μm.

TABLE 2 MORPHOMETRIC ANALYSIS OF ARTERIOLES IN THE WHITE MATTER IN RELATION TO DEMENTIA, DEMYELINATION OF WHITE MATTER AND PRESENCE OF INFARCTS, DILATED PERIVASCULAR SPACES, SENILE PLAQUES AND AMYLOID ANGIOPATHY

No	*Dementia*	*Ratio*	*SD*	*DPS*	*Infarcts*	*Plaques*	*Amyloid*
Normal white matter on Weil-stained sections							
1	–	0.17	0.04	–	–	–	–
2	–	0.18	0.05	–	L	–	–
3	–	0.15	0.05	–	–	–	–
4	–	0.17	0.04	–	C	+	+
5	–	0.16	0.04	+	LL	–	–
6	–	0.17	0.05	–	L	+	–
7	+	0.19	0.06	–	C	+	–
8	–	0.18	0.05	–	–	+	+
Moderate demyelination of the white matter							
9	+	0.25	0.06	+	L	–	+
10	–	0.25	0.05	+	L	–	–
11	–	0.22	0.06	–	–	+	+
12	+	0.27	0.07	–	LL	–	–
Severe demyelination of the white matter							
13	+	0.26	0.06	+	–	–	–
14	+	0.29	0.06	+	LL	–	–
15	+	0.26	0.06	+	L+C	–	–
16	–	0.24	0.06	–	C	+	–
17	–	0.23	0.05	+	–	–	–
18	+	0.25	0.06	+	–	+	–
19	+	0.24	0.06	+	–	+	+

DPS = dilatation of perivascular spaces, L = lacunar infarct; C = cortical infarct.

Dilated perivascular spaces (DPS, fig. 3c) were present in 6 of the 7 brain specimens with severe demyelination and arteriolosclerosis, in 2 of the 4 brains with moderate demyelination and arteriolosclerosis, and in 1 brain specimen with normal white matter and normal arterioles (Table 2). The number of DPS and sum of their measured areas per cm^2 varied widely, with an extremely high number of DPS in 2 brain specimens (fig. 5). The presence and degree of DPS in the total group correlated well ($r = 0.63$, $P < 0.01$) with lower relative brain weight, which is the actual brain weight divided by the reference brain weight according to age and sex (Dekaban and Sadowsky, 1978).

DISCUSSION

We found a strong correlation between lesions of increased signal intensity in the white matter on T_2-weighted images on MRI and the presence of demyelination and gliosis in 19 brain specimens. Demyelination of the white matter was always accompanied by arteriolosclerosis, whereas normal arterioles were always found in brains with normal white matter. Dilatation of perivascular spaces (DPS) was strongly but incompletely correlated with demyelination and arteriolosclerosis. The presence of amyloid angiopathy and senile plaques in the grey matter was not related to demyelination of the white matter.

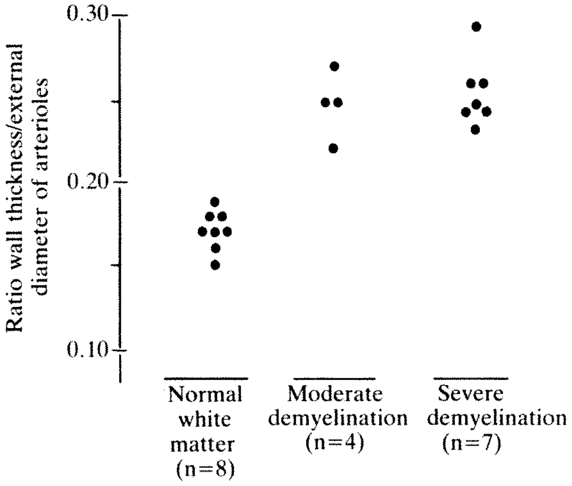

FIG. 4. Correlation of degree of arteriolosclerosis, expressed as the ratio of wall thickness to external vessel diameter of arterioles, with the presence and severity of demyelination in Weil-stained sections in 19 brain specimens.

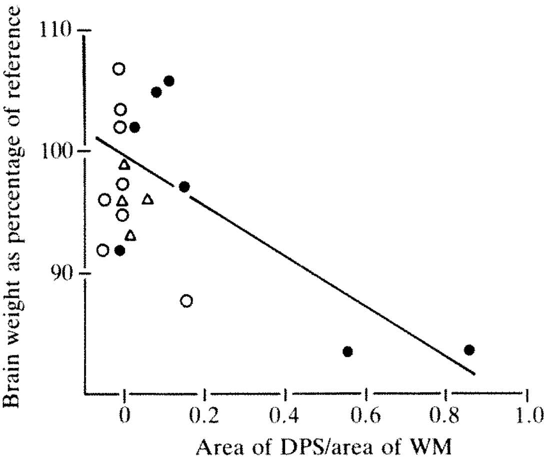

FIG. 5. Sum area of dilated perivascular spaces (DPS) in mm^2 per unit white matter (WM) area in cm^2 correlated with relative brain weight (actual brain weight as a percentage of reference brain weight according to sex and age (Dekaban and Sadowsky, 1978). This correlation was statistically significant. Normal white matter (○); moderate demyelination (△); severe demyelination (●).

These findings are in favour of the notion that extensive demyelination and/or gliosis is the pathological correlate of periventricular white matter lesions seen on MRI, and they also suggest that arteriolosclerosis precedes and probably causes the demyelination and loss of axons, and that dilatation of perivascular spaces is a secondary event.

The presence of areas of hyperintensity on MRI did not completely correspond with the degree of demyelination in Weil-stained sections of 3 brains. The most probable explanation is that MR images were mostly made in the axial plane, whereas the Weil-section were made in the coronal plane; nevertheless, in a few other cases lesions in the coronal MRI plane corresponded well with similar lesions in the axial plane. Some other limitations of the present study have to be pointed out. First, the frequency of white matter lesions in our study is not representative for the general population, because a large number, namely 13, of brains were obtained from psychogeriatic hospitals. The frequency of the lesions differed also from that in other studies (George *et al.*, 1986; Gerard and Weisberg, 1986), because only large lesions were considered suitable for comparison with slices in the frontal plane. Secondly, the calculated ratios for wall thickness of arterioles should not be considered as absolute values, since contraction by fixation changed the real proportions. In other studies, a correction for calculating the true thickness of the tunica media was carried out (Okeda, 1973), or injection of barium sulphate under high pressure into the cerebral vessels was performed (Ross Russell, 1963; Cook and Yates, 1972). Nevertheless, the mean ratios of the arterioles in these 19 brains could be compared with each other, which resulted in a sharp cut-off point with regard to arteriolar size between brains with or without demyelination. The same limitation applies to the summed area of dilated perivascular spaces. Although these spaces could be histologically well differentiated from artefactual spaces as a result of retraction (Woollam and Millen, 1968), they were probably of smaller size in vivo.

We found lesions with increased signal intensity of the white matter on the T_2-weighted images in nearly every brain with demyelination and gliosis. The origin of the changes in signal intensity, i.e., in T_1 and T_2 relaxation times and proton density, cannot be attributed to 'demyelination' per se. Astrocytic gliosis has proven to result in a prolongation of the relaxation time T_1 and a minor change in T_2 (Barnes *et al.*, 1988), and it has been argued that MRI changes seen in multiple sclerosis and Binswanger's disease are caused by increased extracellular water content as well as astrocytic gliosis. In accordance with Révész *et al.* (1989), we consider that the MRI lesions in our formalin-fixed brains are the result of increase of extracellular space due to demyelination, together with gliosis.

CT scanning has proved less sensitive than MRI in demonstrating white matter lesions in series in which it was compared with postmortem studies of white matter (Lotz *et al.*, 1986; Rezek *et al.*, 1987; Janota *et al.*, 1989). Other studies in which MRI scanning in a series of brains of elderly patients was compared with neuropathological investigation resulted in conclusions different from ours. In some of these studies periventricular or focal lesions in the white matter were attributed to DPS or lacunar infarcts (Awad *et al.*, 1986*b*; Braffman *et al.*, 1988), and not primarily to demyelination. But others, like ourselves, explained even punctate lesions around the frontal horns in all investigated brains as demyelination (Sze *et al.*, 1986). Another finding we could not confirm is a putative concurrence of Alzheimer changes or amyloid angiopathy with demyelination (Gray *et al.*, 1985; Brun and Englund, 1986).

In our study, we found infarcts in some but not all patients with demyelination and arteriolosclerosis. Binswanger originally described white matter atrophy without focal lesions in 8 patients with slowly progressive dementia followed by focal neurological deficits. He considered this to be a clinical and pathological entity (Binswanger, 1894). According to Binswanger, the disease should be differentiated from the so-called 'arteriosklerotische Hirndegeneration', multi-infarct dementia in present terms, by the absence of focal lesions. However, this distinction became less clear by Alzheimer's description of focal lesions in similar cases of diffuse demyelination (Alzheimer, 1898). In more recent studies (Caplan and Schoene, 1978; Babikian and Ropper, 1987), the occurrence of minor strokes became even a conditional element in the diagnosis of Binswanger's disease. In that view, Binswanger's disease and the lacunar state frequently coexist and in their pure forms they represent only the two extremes of a pathophysiological continuum. It might also be argued, however, that in Binswanger's disease, whether fully developed or not, the brunt of the disease is borne by small arterioles, below 150 μm in size, whereas lacunar infarcts are usually caused by occlusion of larger penetrating arteries, between 400 μm and 1 mm.

Our method of measuring relative wall thickness correlated exactly with the presence or absence of demyelination. The sharp distinction in arteriolar size between brains with and without demyelination suggests that arteriolosclerosis is soon followed by demyelination; otherwise one would expect to find some brains with arteriolosclerosis but without demyelination. Thickening of walls of cerebral arteries with diminution in lumen has been found with increasing age and hypertension (Ross Russell, 1963; Cook and Yates, 1972). In all postmortem cases of Binswanger's disease, arteriolosclerosis, that is, thickening of arteriolar walls with hyalinosis of the tunica media, has been found (Olszewski, 1962; Caplan and Schoene, 1978). In two studies a semiquantitative correlation was found between arteriolosclerosis and demyelination (Goto *et al.*, 1981; Lotz *et al.*, 1986), which is in accordance with our results.

Does demyelination precede loss of axons or do nerve fibres disappear as a whole? Révész *et al.* (1989) emphasized that in fully developed Binswanger's disease there is a more or less equal loss of axons and myelin, and that the term demyelination is therefore inappropriate. But we found a variable degree of axonal swelling and axon loss within the group with severe demyelination, which suggests that demyelination may indeed be the primary process.

We found that the presence and severity of DPS were strongly correlated with arteriolosclerosis and with loss of brain weight after correction for age. Dilatation of perivascular spaces, together with loss in brain weight, was already mentioned in studies of 'arteriosklerotische Hirndegeneration' (Binswanger, 1894; Alzheimer, 1898). Other authors described these spaces in cases of Binswanger's disease (Nissl, 1920; Garcin *et al.*, 1960). Although DPS should be distinguished from lacunar infarcts (Marie, 1901), they have frequently been found in brains of hypertensive patients (Cole and Yates, 1967). The pathogenesis of these spaces is disputed. The hypothesis of unfolding of small arteries and high pressure pulsation as a cause of DPS, as proposed by Hughes (1965), is supported by the coexistence of arteriolosclerosis in many patients (Cole and Yates, 1967). Ball (1989) defended the view that cortical atrophy was the primary factor, followed by loss of axons and myelin, and finally by formation of DPS 'ex vacuo'. He did not count arteriolosclerosis as a factor. But in contrast to Ball's hypothesis, we found demyelination and arteriolosclerosis in 8 of 9 brains with DPS, whereas senile plaques and amyloid angiopathy were found as often in brains with DPS as in those without. We therefore suggest that demyelination, and not cortical cell loss, precedes the development of DPS. The loss of nerve fibres is most probably the result of arteriolosclerosis with chronic ischaemia (De Reuck *et al.*, 1980). The wide range of severity of DPS indicates a gradual process of demyelination and subsequent cerebral atrophy, which might explain that moderate demyelination in 3 brains and severe demyelination in 1 brain had not yet resulted in significant cerebral atrophy and development of DPS. This explanation does not exclude a more direct role of arterioles in the development of dilated perivascular spaces by pulsatile forces.

Do the findings in our series represent Binswanger's disease, or degrees of it? Diffuse white matter involvement in an elderly patient can be associated with other pathological conditions, such as a diffuse non-Hodgkin lymphoma which was found in one of the present cases (Hochberg and Miller, 1988). Moreover, diffuse white matter lesions in a demented patient do not exclude Alzheimer's disease. Finally, dementia in a patient with periventricular lesions and enlarged ventricles is sometimes, but not always, caused by a normal pressure hydrocephalus (Caplan and Schoene, 1978; Thomsen *et al.*, 1986). Two patients in our series had dementia and severe white matter lesions on postmortem MRI but no infarcts or Alzheimer changes in the cortex, and can be considered as 'pure' examples of Binswanger's disease. Our results therefore confirm previous reports that diffuse demyelination can be the only cause of dementia.

ACKNOWLEDGEMENTS

We are grateful to Dr R. Ravid, of the Netherlands Brain Bank Amsterdam, for providing us with a number of brain specimens, to Mr H. Sakkers for neuropathological staining of brain specimens, to Mr H. Veltman for his help with quantitative investigation of blood vessels, to Dr J. C. van Latum and Dr P. R. Bär for advice on statistics and other comments, and to Dr E. F. M. Wijdicks for reviewing the manuscript. This investigation has been supported by the N. W. O. Council for Medical Research (grant no. 716.006).

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(*Received February 6, 1990. Revised April 24, 1990. Accepted May 2, 1990*)

Brain (1991), 114, 775–788

UBIQUITIN-IMMUNOREACTIVE INTRANEURONAL INCLUSIONS IN AMYOTROPHIC LATERAL SCLEROSIS

MORPHOLOGY, DISTRIBUTION, AND SPECIFICITY

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SUMMARY

Antibodies to ubiquitin have been used to search for evidence of abnormal protein degradation in amyotrophic lateral sclerosis—motor neuron disease (ALS). Anterior horn cell ubiquitin-immunoreactive (IR) inclusions were present in all of 31 ALS cases but in none of 23 neurologically normal and in only 1 of 22 neurologically abnormal controls. These inclusions, which were present in familial and sporadic ALS cases, and in cases with dementia, took the form of dense rounded or irregular ubiquitin-IR cytoplasmic inclusions (dense bodies), or loosely arranged bundles ('skeins') of filamentous-appearing material. The presence of ubiquitin-IR inclusions corresponded to the pattern of selective neuronal vulnerability in ALS, although inclusions in pyramidal neurons of the motor cortex were infrequent and were noted in only a minority of cases. Ubiquitin-IR inclusions were more prevalent than Bunina bodies. The latter were present in 67% of ALS cases but were seldom labelled by antibodies to ubiquitin. Intraneuronal inclusions resembling Lewy bodies were present in 23% of ALS cases and were often identified by antibodies to ubiquitin. We conclude that the presence of ubiquitin-IR inclusions in lower motor neurons represents a characteristic pathological feature of ALS in its various clinical forms. Ubiquitin-IR inclusions in ALS differ from ubiquitinated inclusions in other neuronal degenerations in that they are not readily identified by antibodies to cytoskeletal proteins. They may represent accumulations of altered or abnormal neuronal proteins resistant to degradation via the ubiquitin proteolytic pathway.

INTRODUCTION

Amyotrophic lateral sclerosis (ALS, motor neuron disease) is a progressive motor disorder of unknown cause characterized by degeneration of anterior horn cells, brainstem motor neurons, large pyramidal neurons of the motor cortex, and the lateral and anterior corticospinal tracts (Charcot and Joffroy, 1869; Bertrand and Van Bogaert, 1925). Cytoplasmic intraneuronal inclusions are a major pathological feature of other neurodegenerative disorders, especially Alzheimer's disease (AD), Parkinson's disease

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(PD) and Pick's disease, and identification of the molecular composition of these inclusions is one approach to clarifying the biochemical processes leading to cell death in these disorders (Goldman and Yen, 1986). It has recently been found that neurofibrillary tangles (NFTs), Lewy bodies and Pick bodies react with antibodies against ubiquitin, a low molecular weight protein implicated in the nonlysosomal degradation of short-lived and abnormal proteins (Cole and Timiras, 1987; Mori *et al.*, 1987; Perry *et al.*, 1987; Kuzuhara *et al.*, 1988; Lennox *et al.*, 1988; Love *et al.*, 1988; Lowe *et al.*, 1988*a*; Leigh *et al.*, 1989*a*). This suggests that proteins in these structures are relatively resistant to proteolysis via the ubiquitin pathway.

In ALS, a variety of neuronal inclusions have been described. The most specific is the Bunina body, which is a small (1 – 10 μm) eosinophilic cytoplasmic inclusion found in anterior horn cells in up to 70% of familial and sporadic cases (Bunina, 1962; Chou, 1979). Other neuronal inclusions such as Lewy body-like inclusions (Hirano *et al.*, 1967, 1984; Takahashi *et al.*, 1972; Kuroda *et al.*, 1986; Delisle *et al.*, 1987; Kato *et al.*, 1988; Kusaka *et al.*, 1988), basophilic bodies (Nelson and Prensky, 1972; Oda *et al.*, 1978; Chou, 1979), hyaline bodies (Schochet *et al.*, 1969; Kondo *et al.*, 1986) and at the ultrastructural level, tubular and granular particles and neurofilament accumulations (Norris *et al.*, 1975; Oda *et al.*, 1978; Averback, 1981; Kondo *et al.*, 1986) are less commonly seen. Rarely, neurofibrillary tangles have been noted in anterior horn cells in Guam ALS-parkinsonism-dementia syndrome (Hirano *et al.*, 1966). Unlike inclusions in other neuronal degenerations, the Bunina body is not labelled by antibodies against neurofilaments (Leigh *et al.*, 1988, 1989*b*) and its molecular composition is unknown. The observation that neuronal inclusions in AD, PD, and Pick's disease are labelled by antibodies to ubiquitin prompted us to search for evidence of abnormal protein degradation and persistent ubiquitin-protein conjugates in ALS motor neurons. We found characteristic ubiquitin-IR inclusions in lumbar spinal cord anterior horn cells in 10 ALS cases but in none of 12 control cases examined, and suggested that these inclusions may represent ubiquitin conjugated to unidentified protein(s) which are resistant to degradation via the ubiquitin pathway (Leigh *et al.*, 1988). Others have independently reported similar findings (Chou, 1988). We now report a detailed study of the morphology, distribution, and specificity of ubiquitin-IR inclusions in a large series of ALS and neurologically normal and abnormal control cases, including those cases in which the lumbar spinal cord was studied previously (Leigh *et al.*, 1988). The present material includes patients with all clinical forms of the disease. Our observations show that a characteristic molecular abnormality is present in vulnerable lower motor neurons in sporadic and familial ALS and in ALS with dementia, and support the notion that similar or identical pathogenic mechanisms underlie these variants of the ALS syndrome.

METHODS

For light microscope studies, tissue was fixed in 10% buffered formalin, embedded in paraffin wax, and sectioned serially at 5 – 10 μm. Spinal cord was available from 31 cases of ALS (Table 1) although full clinical and autopsy details were available for 20 of these in whom the clinical features fulfilled the criteria of Li *et al.* (1986). In all cases pathological examination revealed the typical features of ALS. There were 5 cases in which the clinical syndrome of progressive bulbar palsy (PBP) predominated, 13 cases with a combination of muscle wasting and fasciculation with corticospinal tract signs (classical ALS) and 2 cases with the syndrome of progressive muscular atrophy (PMA). There was a positive family history

TABLE 1. ALS CASES CLINICAL DETAILS

Case	*Sex*	*Age (yrs)*	*FH*	*Duration of symptoms*	*Clinical features*	*BBs*	*LB-LIs*
1	F	62	−	1 yr	PBP→ALS	+	−
2	M	69	−	4 yrs	ALS	−	+
3	F	73	−	9 mos	PBP-ALS	+	+
4	F	65	−	A few mos	Vent. failure	+	−
5	F	46	−	9 mos	ALS	+	−
6	M	48	−	2 yrs	ALS	+	−
7	F	75	−	7 yrs	ALS	+	−
8	F	37	−	4 yrs	MS, ALS	+	−
9	M	57	+	2 yrs	PBP→ALS	−	−
10	F	58	+	2 yrs	ALS/D	+	−
11	F	74	−	2 yrs	ALS	+	+
12	M	74	?	?	?	+	−
13	?	?	?	?	?	+	−
14	?	?	?	?	?	+	−
15	?	?	?	?	?	+	+
16	?	?	?	?	?	+	−
17	?	?	?	?	?	−	−
18	?	?	?	?	?	+	−
19	F	69	−	2 yrs	ALS	−	−
20	M	67	−	6 mos	ALS	−	+
21	F	72	−	3 yrs	PBP	+	−
22	M	30	+	6 mos	PMA	−	−
23	F	75	−	3 yrs	ALS/D	−	−
24	M	60	−	8 mos	PBP	−	−
25	M	?	?	?	?	−	−
26	?	?	?	?	?	+	−
27	F	72	−	3 yrs	ALS/D	−	−
28	M	79	−	9 yrs	ALS	−	−
29	?	?	?	?	?	+	+
30	F	59	−	2 yrs	ALS	+	+
31	M	73	+	4 yrs	PMA	+	−

BB = Bunina body, D = dementia; FH = family history, LB-LI = Lewy body-like inclusion; MS = multiple sclerosis, PBP = progressive bulbar palsy; PMA = progressive muscular atrophy; PMD = delay between death and autopsy.

in 4 cases, 2 of whom were siblings (Cases 9, 10); both presented with ALS, but the sister became demented as her illness progressed, whereas there was no evidence of dementia in the brother. In the other 2 cases, the inheritance was probably autosomal dominant, since in each case a parent had died of ALS, and in 1 (Case 31) a brother had also died of ALS. Tissue from some of these cases has been used in a previously reported study (Swash *et al.*, 1986). Dementia was documented in 3 cases and in 2 (Cases 23, 27) this was of frontal lobe type (Neary *et al.*, 1990).

Material from the spinal cord, and in some cases brainstem and cerebral hemisphere, was available from 23 neurologically normal cases including 16 patients in whom death was due to acute myocardial ischaemia, and others with a variety of acute and chronic disorders, including sepsis, pneumonia, carcinomatosis, and status asthmaticus; none had clinical or pathological evidence of neurological illness. Material from the brainstem and cerebral hemispheres was available from a further 6 neurologically normal control cases. Material was available from 22 additional patients in whom death had occurred from neurological disorders other than ALS (Table 2).

The median age of ALS cases was 68 (range 31−79) yrs and of controls 69 (range 4−89) yrs. The median delay from death to autopsy was 24 h in the 16 ALS cases where this information was available, and 27 h in controls. In the ALS group (Table 1) there were 11 males and 13 females; in 7 cases the sex was unknown. In the control series, there were 24 males and 23 females; in 4 cases the sex was unknown.

TABLE 2 NEUROLOGICALLY ABNORMAL CONTROLS CLINICAL AND PATHOLOGICAL DETAILS

Case	*Sex*	*Age (yrs)*	*Clinical diagnosis*	*Neuropathological features*
1	M	44	MI; cerebral anoxia seizures; coma after resuscitation	Chromatolysis of lumbar AHCs
2	F	37	Intracranial haemorrhage	Subarachnoid haemorrhage Normal spinal cord
3	M	47	Friedreich's ataxia	Degeneration of posterior and lateral columns; no definite loss of AHCs
4	M	62	Severe polyradiculopathy; NIDDM, Cor pulmonale	Loss of AHCs, gliosis of spinal grey matter
5	F	69	IHD, PVD; amputation of right leg 1982	Loss of AHCs from S1 right anterior horn
6	M	?	Carcinomatous meningitis	Infiltration of leptomeninges with adenocarcinoma cells No loss of AHCs
7	F	74	Lewy body PD	Normal spinal grey matter
8	M	76	Lewy body PD	Normal spinal grey matter
9	F	70	Lewy body PD	Normal spinal grey matter
10	F	72	Lewy body PD	Normal spinal grey matter
11	M	79	Akinetic-rigid syndrome ?MSA	No loss of AHCs; Ub-IR inclusions in some AHCs
12	F	86	Alzheimer's disease	Normal spinal grey matter Many tangles and plaques in HC
13*	M	74	Progressive supranuclear palsy	Normal spinal grey matter
14	F	60	Huntington's disease	Cortex, basal ganglia, and brainstem examined; spinal cord not available
15	F	4	Werdnig-Hoffman disease	Marked depletion of AHCs with gliosis of grey matter
16	F	36	Multiple sclerosis	Normal AHCs; demyelination of posterior, lateral and anterior tracts
17	F	?	Dialysis dementia	Normal AHCs
18	?	?	Tetanus	Normal AHCs
19	?	?	Guillain-Barré syndrome	Normal AHCs
20	F	67	Tabes dorsalis	Normal AHCs
21**	M	56	Long-standing poliomyelitis	Depletion of lumbar AHCs
22	F	13	Guillain-Barré syndrome	Normal AHCs; inflammatory infiltration of nerve roots

* From Pathology Institute, Basel; ** from Midland Centre for Neurosurgery and Neurology AHC = anterior horn cell; IHD = ischaemic heart disease; MI = myocardial infarction, MSA = multiple system atrophy; NIDDM = non-insulin dependent diabetes mellitus; PSP = progressive supranuclear palsy; PVD = peripheral vascular disease

Serial sections from cervical (C7 or C8), thoracic (T2–4), lumbar (L3–5) and sacral (S1–2) spinal cord and from brainstem and cerebral hemispheres (Table 3) were stained with haematoxylin and eosin (H and E), cresyl violet-luxol fast blue, or processed for immunocytochemistry by the indirect immunoperoxidase method (Anderton *et al.*, 1982; Leigh *et al.*, 1988). Antibodies used included an affinity purified rabbit antibody to ubiquitin prepared and characterized as previously described (Leigh *et al.*, 1988) and a monoclonal antibody (MAb) 147 which recognizes phosphorylated neurofilament epitopes (Miller *et al.*, 1986).

S2 segment anterior horn cells from 5 representative ALS cases and 5 control cases were analysed quantitatively for the presence of ubiquitin-IR inclusions and for the presence or absence of well-defined Nissl substance in immunostained sections counterstained with Mayer's haematoxylin. In this study, split-cell errors were avoided by counting and analysing neurons containing an identifiable nucleolus (Konigsmark, 1970). S2 was chosen for these analyses because neuronal loss was often relatively mild compared with cervical segments. We also attempted to define the relationship between ubiquitin-IR inclusions and cytopathological abnormalities of ALS anterior horn motor neurons by analysing paired sections alternately labelled with ubiquitin antibodies or stained with H and E, allowing us to correlate the presence of Bunina

TABLE 3 SUMMARY OF PATHOLOGICAL MATERIAL EXAMINED AND PRESENCE OF Ub-IR INCLUSIONS (Ub-IRI +)

	ALS cases *(n = 31)*	*Ub-IRI+*	*Control cases* *(n = 51)*	*Ub-IRI+*
Spinal cord	31	31	45	1*
Cervical	27	25	23	1*
Thoracic	14	4	19	0
Lumbar	26	22	39	1*
Sacral	15	12	19	1*
Cerebral cortex				
Motor	9	3	6	0
Temporal	5	0	4	0
Frontal	5	0	2	0
Other areas	5	0	2	0
Brainstem				
Hypoglossal nucleus	15	10	13	0
Oculomotor nucleus	5	0	6	0

* Case 11, Table 2.

bodies, Lewy body-like inclusions, hyaline bodies and other cytopathological abnormalities with ubiquitin-IR inclusions. Finally, sections with motor neurons containing eosinophilic or other inclusions were photographed, decolourized in acid alcohol, and reprocessed for immunocytochemistry with ubiquitin antibodies.

RESULTS

General observations

In ALS cases there was marked depletion of anterior horn cells which was particularly striking in the cervical spinal cord, and usually also in lumbar and sacral spinal cord. True chromatolysis was rarely seen, but achromasia (paucity or absence of normal Nissl substance without swelling of the neuron or displacement of the nucleus to the periphery of the cell) was common. Bunina bodies, defined as small (1 – 10 μm) round, oblong, single or branched eosinophilic cytoplasmic inclusions in anterior horn cells (Bunina, 1962; Chou, 1979) were seen in 20 ALS cases (65%) but in none of the controls (Table 1). Other eosinophilic or basophilic inclusions, mainly in the form of Lewy body-like inclusions, were seen in 7 cases (23%) and, in 5 of these, Bunina bodies were present in other anterior horn cells (Table 1). Lewy body-like inclusions were 10 – 25 μm in diameter, slightly basophilic or achromasic, often with a pale irregular halo and a vesicular core.

Immunocytochemistry with antibodies to ubiquitin revealed anterior horn cell inclusions in all ALS cases (Table 3). These inclusions consisted either of irregular, filamentous 'skein-like' cytoplasmic accumulations (fig. 1A) or dense, compact, rounded or irregular bodies, 5 – 25 μm in diameter within the cytoplasm of anterior horn cells (fig. 1B). Skeins were more common but in some cases only dense accumulations were present. Skeins sometimes extended into the dendrites of motor neurons. In some neurons very fine ubiquitin-IR strands could be seen in the cytoplasm of the perikaryon or dendrites (fig. 1A). Ubiquitin-IR skeins were not identified in adjacent haematoxylin and eosin (fig. 2A, B) or LFB-CV stained sections, or in sections immunostained with antibodies to neurofilament proteins.

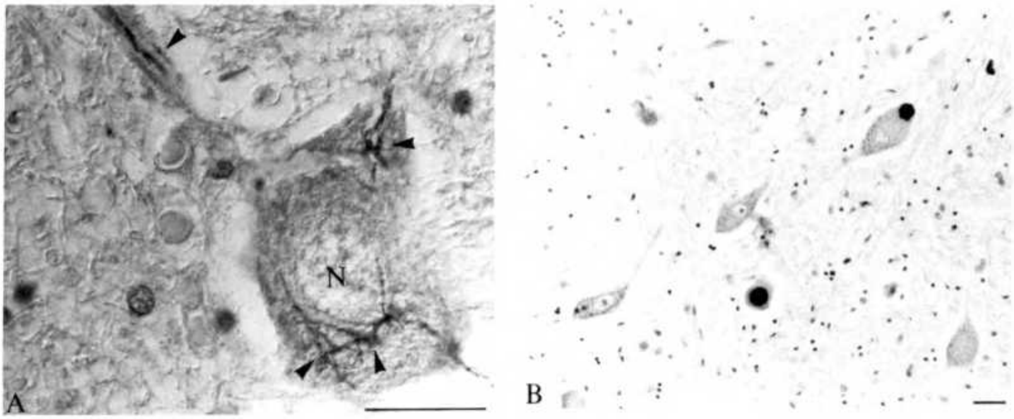

FIG. 1. A, lumbar anterior horn cell from ALS *Case 3* (Table 1) showing skein-like ubiquitin-IR inclusions (arrows) extending into the dendrites. N = nucleus. Nomarski interference optics. B, lumbar anterior horn cells from same case showing ubiquitin-IR dense bodies in 3 anterior horn cells. Bars = 10 μm.

Ubiquitin-IR inclusions were noted in motor neurons at all levels of the spinal cord (Table 3). They were not seen in neurons of the intermediolateral cell column, in cells of Clarke's column, or in dorsal horn neurons. Inclusions were not seen in neurons of Onuf's nucleus in the S2 segment. This nucleus was strikingly spared when compared with adjacent S2 anterior horn cells.

In the 5 ALS cases in which neurons in S2 segment were counted, the prevalence of ubiquitin-IR inclusions was 17% (Table 4). In comparison with 5 normal controls, there was no significant loss of (non-Onuf) sacral anterior horn cells. There was a statistically significant association between the presence of inclusions and the absence of Nissl substance in sacral anterior horn cells (Tables 4, 5). In a survey of 2057 L4 anterior horn cells from the ALS cases in which adjacent H and E and ubiquitin immunolabelled sections were studied, 185 (8.5%) contained ubiquitin-IR inclusions. Of these 185, 132 were unequivocally identified in each of 2 adjacent sections stained with H and E; 92 of these neurons contained skeins and 40 contained dense bodies. Amongst the 92 neurons containing ubiquitin-IR skeins, 14 (15%) contained Bunina bodies and 7 (8%) Lewy body-like inclusions. In the 40 neurons which contained ubiquitin-IR dense bodies, Bunina bodies were identified in 9 (23%) and Lewy body-like inclusions were present in 7 (18%).

In a further series of H and E stained sections in which Bunina bodies or other inclusions were identified and which were subsequently processed for immunocytochemistry with antibodies to ubiquitin, only 2 of 9 Bunina bodies were weakly labelled (fig. 2C, D). In contrast, 7 of 8 Lewy body-like inclusions were strongly immunolabelled. Some showed central immunolabelling (fig. 3A, B) whereas others showed an unlabelled central zone with surrounding ubiquitin-IR skein-like structures (fig. 3C, D). Neither Bunina bodies nor Lewy body-like inclusions were identified by neurofilament or other cytoskeletal antibodies.

Sections from the medulla were examined in 15 ALS and 13 control cases, and from midbrain in 5 ALS and 6 control cases; multiple sections from other areas of brainstem

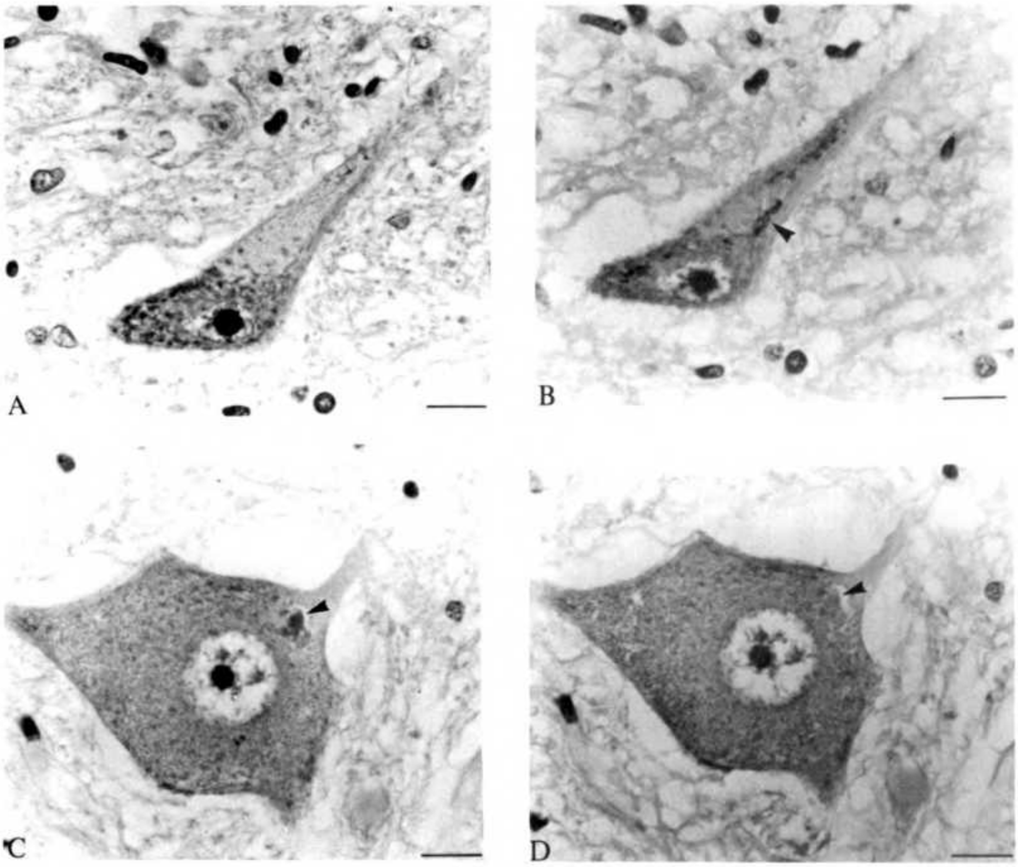

FIG. 2. A, normal appearing ALS lumbar anterior horn cell. (H, E). B, same section and cell, reprocessed for immunocytochemistry with antibodies to ubiquitin, revealing Ub-IR skeins (arrows). C, ALS lumbar anterior horn cell from *Case 31* (Table 1) showing Bunina body (arrow). D, same section and cell, reprocessed for immunocytochemistry with antibodies to ubiquitin. Bunina body is not labelled (arrow). Bars = 10 μm.

TABLE 4. NUMBER OF ANTERIOR HORN CELLS CONTAINING NUCLEOLI IN SECTIONS FROM S2 SEGMENT IN 5 ALS CASES AND 5 CONTROLS

	Total no. of nucleoli	*Mean no. (±SD) per section*
ALS	117	5.8 (3.5)
Controls	125	6.1 (2.85)

were examined in 5 ALS and 5 control cases (Table 3). Ubiquitin-IR inclusions were noted in motor neurons of the hypoglossal nuclei, accessory nucleus, nucleus ambiguus, facial, and trigeminal motor nuclei in ALS cases but in none of the control cases examined. Rare inclusions were noted in oculomotor neurons (fig. 4) which was well preserved

TABLE 5. ASSOCIATION BETWEEN UBIQUITIN-IMMUNOREACTIVE INCLUSIONS (Ub-IRI) AND PRESENCE (+) OR ABSENCE (−) OF NISSL SUBSTANCE IN ANTERIOR HORN CELLS*

	Ub-IRI present		*UB-IRI absent*		
Observations	*Observed*	*Expected*	*Observed*	*Expected*	*Total*
Nissl (+)	128	166	869	831	997 (90%)
Nissl (−)	57**	19	57	95	114 (10%)
Total observed	185 (17%)		926 (83%)		1111 (100%)

* Observations from 5 μm sections of S2 segment from 5 ALS cases. Table gives observed and expected numbers assuming null hypothesis, i.e., no correlation between presence or absence of Nissl and presence or absence of Ub-IRI.
** $P < 0.001$, χ^2 test.

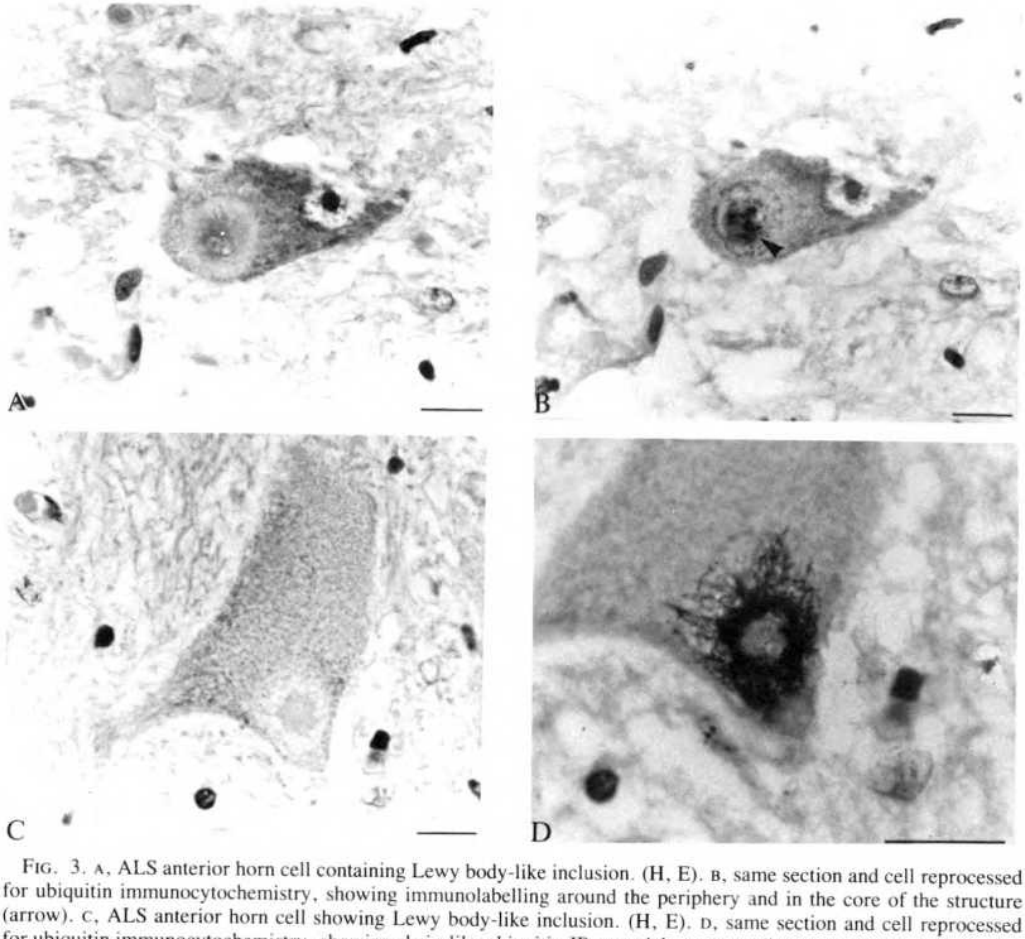

FIG. 3. A, ALS anterior horn cell containing Lewy body-like inclusion. (H, E). B, same section and cell reprocessed for ubiquitin immunocytochemistry, showing immunolabelling around the periphery and in the core of the structure (arrow). C, ALS anterior horn cell showing Lewy body-like inclusion. (H, E). D, same section and cell reprocessed for ubiquitin immunocytochemistry, showing skein-like ubiquitin-IR material corresponding to the 'halo', surrounding an unlabelled amorphous core. Bars = 10 μm.

in comparison with hypoglossal motor neurons although the extent of cell loss in the latter was variable. In the motor cortex, rare granular or filamentous ubiquitin-IR structures were noted in large pyramidal neurons in 3 cases examined (fig. 5A;

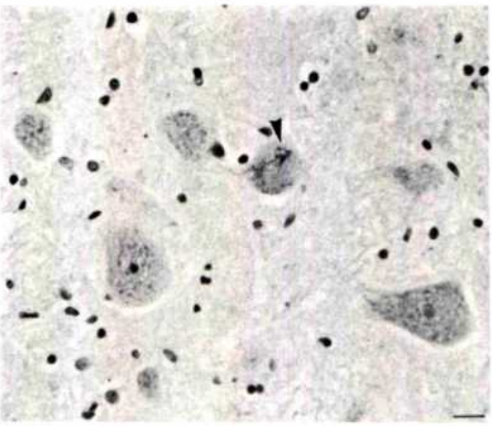

FIG. 4. Oculomotor nucleus showing ubiquitin-IR skeins in one motor neuron (arrow). Bar = 10 μm.

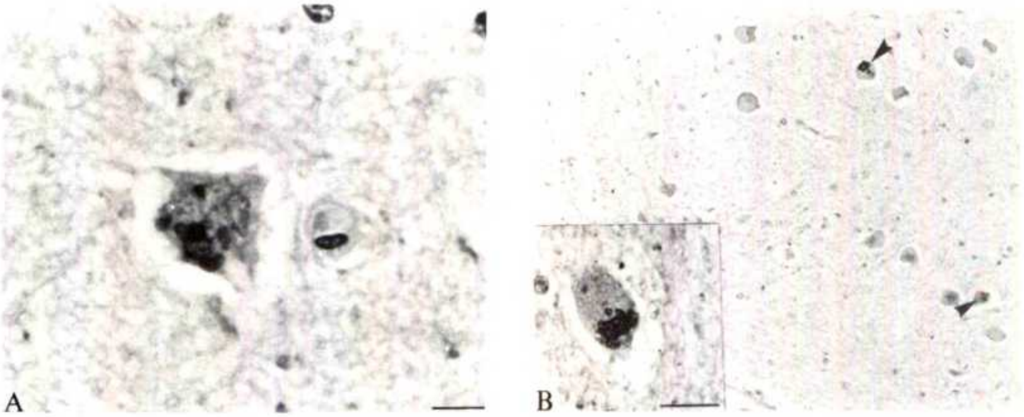

FIG. 5. A, cortical neuron from ALS *Case 3* showing granular faint skein-like ubiquitin-IR structures. B, ubiquitin-IR inclusions (arrows) in neurons of the inferior olivary nucleus in a control case. Bars = 10 μm.

Cases 3, 7, 9, Table 1), but the majority of motor cortex neurons failed to show either diffuse antiubiquitin staining or inclusions.

Skein-like and rounded ubiquitin-IR anterior horn cell inclusions were seen in a few cervical, lumbar and sacral anterior horn cells in 1 neurological control case (Table 2, Case 11). This was a man who died at the age of 69 yrs with an 8 yr history of dysarthria, dysphagia, ataxia, rigidity, and supranuclear ophthalmoplegia but without clinical or pathological features of ALS. No ubiquitin-IR anterior horn cell inclusions were seen in 4 cases of Parkinson's disease associated with brainstem Lewy bodies (Table 2, Cases 7−10). Anterior horn cells disorders, including poliomyelitis (Table 2, Case 21) and of spinal muscular atrophy type (Table 2, Case 15) were not associated with ubiquitin-IR inclusions. We were not able to examine material from acute poliomyelitis, or from patients with 'postpoliomyelitis' deterioration. We did, however, have material from 1 patient (Table 1, Case 30) who at the age of 32 yrs had developed severe paralytic poliomyelitis affecting the limbs but sparing bulbar muscles. She remained stable for 26 yrs, but at the age of 58 she developed progressive limb weakness and fasciculations, progressing to bulbar weakness and death over 2 yrs. Clinically, this was thought to be ALS rather than postpoliomyelitis deterioration. Many of the surviving anterior horn cells contained typical ubiquitin-IR inclusions.

Ubiquitin-IR structures common to both ALS and control tissue included axonal spheroids in the spinal cord, which showed a granular or punctate pattern of immunoreactivity, and small dots in white matter similar to the ubiquitinated deposits described in normal senescent brain by Pappolla *et al.* (1989). In addition, we observed lobulated ubiquitin-IR inclusions in the neurons of the inferior olivary nucleus in ALS and in control cases (fig. 5B). This was the only site in which intraneuronal ubiquitin-IR inclusions were observed in neurologically normal controls, and the only site other than motor neurons in which intracellular inclusions were seen in ALS cases.

DISCUSSION

Our studies confirm the distinctive nature of ubiquitin-IR inclusions found in ALS anterior horn cells and show that they also occur in cranial nerve motor neurons, and occasionally in cortical motor neurons (Leigh *et al.*, 1988). They are thus closely associated with the characteristic pathological features of ALS, namely degeneration of motor neurons in the cerebral cortex, cranial motor nuclei and spinal anterior horns. These intraneuronal inclusions are much more prevalent in the brainstem and spinal cord than in the motor cortex, where we encountered only rare filamentous or granular ubiquitin-IR accumulations. Elsewhere in the nervous system, the presence of ubiquitin-IR inclusions reflects the well-documented selective vulnerability of motor neuron involvement in ALS (Lawyer and Netsky, 1953; Brownell *et al.*, 1970) with inclusions frequently encountered in the hypoglossal nuclei but rarely seen in the well-preserved oculomotor nuclei. Although we did not see inclusions in Onuf's nucleus, sparing of this nucleus is not invariable in ALS, and the presence of inclusions may correlate with incontinence.

These ubiquitin-IR inclusions were characteristic of ALS and were seen in all our cases. They may not be entirely specific, since we have noted similar inclusions in 1 control case with atypical parkinsonism. In this case there was a loss of pigmented substantia nigra neurons without Lewy bodies or tangles suggestive of progressive supranuclear palsy. Intermediolateral cell columns and anterior horn motor neurons were normal in number and appearance, apart from occasional chromatolytic neurons. The available evidence suggests that ubiquitin-IR anterior horn cell inclusions represent a distinctive pathological feature of ALS (Chou, 1988; Leigh *et al.*, 1988).

The inclusions were morphologically similar in cases representing all the clinical syndromes of ALS, including progressive muscular atrophy, which was the form of the disease in 2 of our 4 familial cases with a history suggestive of autosomal dominant inheritance. The presence of this distinctive marker in familial, sporadic and demented cases implies common mechanisms for the formation of the inclusions and thus of the neurodegenerative process itself.

Ubiquitin-IR skeins were more common than ubiquitin-IR dense bodies, as we reported previously (Leigh *et al.*, 1988). We had the impression that the latter are composed of aggregated skeins since inclusions in some neurons appeared to represent transitional forms. Our observations suggest a sequence of events leading from the formation of delicate strands of ubiquitin-IR filamentous material in the cytoplasm of normal-appearing anterior horn cells, to aggregations of filaments into skeins (and dense bodies) which are often associated with other cytopathological abnormalities such as loss of Nissl substance, vacuolar change, Lewy body-like inclusions, basophilic bodies and Bunina bodies (Chou, 1979). Ubiquitin-IR inclusions were found in 17% of sacral anterior horn cells in a group of ALS cases in which there was no significant loss of sacral anterior horn cells, suggesting that the ubiquitin-IR inclusions are not simply markers of the final stages of cell damage. Bunina bodies, however, probably develop at a relatively late stage of neuronal degeneration, since most neurons containing Bunina bodies lack normal Nissl substance and appear achromasic. We have shown that Bunina bodies themselves are usually not ubiquitinated, although they are often associated with ubiquitin-IR material (Chou, 1988; Lowe *et al.*, 1988*b*).

Lewy body-like inclusions have been described in familial and sporadic ALS (Hirano *et al.*, 1967; Takahashi *et al.*, 1972; Chou, 1979; Hirano *et al.*, 1984; Kuroda *et al.*, 1986; Delisle *et al.*, 1987; Chou, 1988; Kusaka *et al.*, 1988; Kato *et al.*, 1989; Sasaki *et al.*, 1989). Our findings are in keeping with those of Chou (1979) and suggest that Lewy body-like inclusions are relatively common in ALS, and that they can be observed in all clinical varieties of ALS. Unlike classical brainstem Lewy bodies, Lewy body-like inclusions in ALS anterior horn cells are not identified (in wax-embedded tissue) by antibodies to neurofilaments, although they are usually strongly labelled by ubiquitin antibodies (Kato *et al.*, 1989; Sasaki *et al.*, 1989). In this respect, they are similar to cortical Lewy bodies (Lennox *et al.*, 1989). Lewy body-like inclusions in the spinal cord often show an irregular pale halo traversed with fine strands of material which correspond to skein-like ubiquitin-immunoreactive material. Others have a homogeneous or granular eosinophilic or basophilic appearance with H and E and correspond to ubiquitin-IR dense bodies. Ultrastructural studies of Lewy body-like inclusions indicate that they are rather variable in composition. Most are composed of randomly arranged linear structures associated with ribosome-like particles and collections of 10 or 13–25 nm diameter filamentous structures, but some inclusions contain vesicles or granular material with or without filamentous structures (Takahashi *et al.*, 1972; Hirano *et al.*, 1984; Kusaka *et al.*, 1988; Sasaki *et al.*, 1989). Identification of their molecular composition will be important in defining their relationship to Lewy bodies and pale bodies (Leigh *et al.*, 1989*a*) in Parkinson's disease.

Some axonal spheroids in ALS and controls show granular or punctate ubiquitin immunoreactivity (Leigh *et al.*, 1988). This observation suggests that spheroids contain altered proteins which are targets for ubiquitin, although neither the presence of spheroids nor their labelling by ubiquitin antibodies is specific for ALS. Spheroids in ALS and control cases are intensely labelled by antibodies against phosphorylated neurofilament epitopes (Schmidt *et al.*, 1987; Manetto *et al.*, 1988; Leigh *et al.*, 1989*b*; Toyoshima *et al.*, 1989) but there is no direct evidence that altered neurofilament proteins are targets for ubiquitin.

Apart from the presence of ubiquitin, the molecular composition of ubiquitin-IR inclusions in ALS is unknown. It has been suggested that in neurofibrillary tangles in Alzheimer's disease ubiquitin may be conjugated to abnormal protein(s) but that this conjugate is resistant to degradation via the ubiquitin proteolytic pathway (Perry *et al.*, 1987). Ubiquitin-IR inclusions in ALS differ from inclusions in other neurodegenerative disorders in that they are not labelled by antibodies to cytoskeletal proteins such as neurofilaments, actin, or tau although they may be associated with tubulin (Chou, 1988; Leigh *et al.*, 1988). Nevertheless, it is likely that the filamentous structures associated with ubiquitin immunoreactivity in ALS (Lowe *et al.*, 1988*b*; Sasaki *et al.*, 1989) represent stages in the breakdown and degeneration of neurofilament or other cytoskeletal proteins.

In summary, characteristic ubiquitin-IR inclusions are present in vulnerable lower motor neurons in all cases of ALS examined. They are occasionally seen in cortical neurons in the motor area. Although they seem not to be entirely specific for ALS, they provide a valuable molecular marker for the histopathological diagnosis of the disease, since they are much more abundant than Bunina bodies or Lewy body-like inclusions. We have not yet been able to identify a specific cytoskeletal or other protein in association with ubiquitin-immunoreactivity. Identification of the protein conjugate of ubiquitin in ALS is likely to provide a clue to the mechanism of neuronal damage in this disorder.

ACKNOWLEDGEMENTS

This work was supported by the Motor Disease Association of Great Britain, The Wellcome Trust, the MRC and by Mr J. C. Lewis. J.E.M. holds an MRC Training Fellowship. We are grateful to Dr I. Janota, Dr Martin Cook, Dr Rufus Crompton, Professor S. Lieberman and Dr K. Cummings for access to pathological material, to the neurologists at Atkinson Morley's Hospital, London, for providing clinical information on many of the cases, and to the patients, relatives and staff of St Christopher's Hospice, London, for their encouragement and help.

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(*Received January 9, 1990. Revised April 17, 1990. Accepted May 7, 1990*)

ADDENDUM

Since this paper was submitted, Kato *et al.* (Kato S, Hirano A, Suenaga T, Yen S-H (1990) Ubiquitinated eosinophilic granules in the inferiory olivary nucleus. *Neuropathology and Applied Neurobiology*, **16**, 135–139) reported the presence of ubiquitinated intracytoplasmic eosinophilic granules in the inferior olivary neurons of 99 normal individuals. Their number increased with age, and was not increased in Alzheimer's disease or spinocerebellar degeneration.

Brain (1991), **114**, 789–800

A HISTORIC CASE OF VISUAL AGNOSIA REVISITED AFTER 40 YEARS

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SUMMARY

In one of the seminal works on visual agnosia, Adler (1944, 1950) presented the case of a 22-yr-old woman who sustained carbon monoxide cerebral toxicity in the Cocoanut Grove nightclub disaster of 1942. We located this patient 40 yrs after injury and performed a detailed reevaluation. The patient demonstrated persistent deficits in visual recognition, characterized most prominently by defective recognition of elemental shape and form, associated with alexia, prosopagnosia, visuospatial disorientation and impaired visual imagery. Visual acuity, colour recognition, writing ability and verbal intelligence were relatively preserved. Isolated bilateral occipital injury was demonstrated by CT and MRI scanning. On comparison with previously reported cases, our results support the hypothesis that carbon monoxide toxicity can induce a visual agnosia of the apperceptive type with well defined characteristics, seldom seen with other types of cerebral injury. Prognosis for long-term recovery is poor.

INTRODUCTION

The acquired disorders of visual recognition include visual agnosias of several forms and alexia without agraphia occurring in isolation ('pure alexia'). These syndromes are rare and elucidation of their clinical and pathogenetic features has been dependent upon relatively few well described case reports.

In one of the seminal works on visual agnosia, Alexandra Adler (1944, 1950) described a 22-yr-old woman who developed a profound disorder of visual recognition as the result of injury sustained in the Cocoanut Grove nightclub disaster of 1942. Unusual features reported by Adler include the aetiology, carbon monoxide toxicity, which may be responsible for a unique form of visual agnosia (Benson and Greenberg, 1969); the presence of alexia without agraphia with preserved visual fields, rarely encountered in cases of pure alexia (Greenblatt, 1973); and the lack of confounding aphasia or dementia, allowing for detailed analysis of a visual integrative disturbance in a relatively pure form. As one of the earliest and most meticulously documented studies of a visual agnosia of the apperceptive type, Adler's work has had significant influence on subsequent investigators of disorders of complex visual processing.

As the long-term natural history of visual agnosia has not been well characterized, and the localization and neuropsychological mechanism of the apperceptive form remain incompletely understood, we located Adler's patient 40 yrs after injury, replicated many of the studies that had been previously performed and performed additional

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neuropsychometric, radiological and electrophysiological testing unavailable at the time of her earlier evaluations. Our results demonstrate continued fixed deficits in visuospatial perception and, despite normal intelligence and high motivation, minimal improvement in her alexia in the intervening years. We describe unique aspects of carbon monoxide-induced visual agnosia in comparison with other previously reported cases.

CASE HISTORY

H.C. was a healthy, right-handed 22-yr-old woman, a high school graduate employed as a comptometer operator, when she attended the Cocoanut Grove nightclub on the evening in November 1942, when a tragic fire claimed 491 lives. She lost consciousness while trying to escape and was rescued by firemen after at least 20 min of exposure to fumes. On arrival at Boston City Hospital she was conscious, but was agitated, confused, and apparently blind. She had mild respiratory difficulty, hoarseness, and a minor burn on the right shoulder. She remained confused for several days with flight of ideas and inappropriate elation. Her speech remained fluent throughout. She had poor memory, including amnesia for the fire. She did not fix her eyes on any target and gazed straight ahead for prolonged periods. She was able, however, to recognize objects and people by tactile, auditory and olfactory sensations (e.g., she distinguished physicians from nurses by the smell of tobacco on the physicians' clothing). A neuropsychologist found normal optic fundi, reactive pupils, and normal sensory and motor examinations. His initial impression was that the patient's 'blindness' was a form of hysteria.

On the fifth hospital day the patient was examined by Dr Alexandra Adler of the Boston City Hospital Neurological Unit. Adler concluded that her symptoms were due to brain injury, and began a series of investigations that were to span 5 yrs. On her initial evaluation Adler found that she could distinguish light from dark, but could not recognize form or colour. After 2 wks she could name and match colours by intensity and hue, and visual acuity was normal.

With return of normal visual acuity the patient exhibited a profound alexia, initially unable to identify any word or single letter. On the 17th hospital day she recognized the letter H, but no others, and inferred her name, HELEN, from the first letter and length of the word. She subsequently discerned other letters by recognition parts, frequently confusing letters of similar form. After 6 months she was able to read printed words, but not handwriting, even her own. Five years after injury she was able to read a novel, but found reading to be a slow laborious process, devoid of pleasure. Her ability to read handwriting remained impaired.

In addition to this alexia, Adler documented a pervasive underlying visual agnosia. For the first 2 wks she was unable to recognize any object presented visually. Over the next month she began to perceive the outer contours of objects and, ultimately, began to discern internal detail. By 6 months visual object agnosia could be demonstrated only tachistoscopically. She experienced difficulty in recognizing faces of well-known acquaintances on first encounter after injury, and devised a system for recalling faces by memorizing salient features. She was unable to copy simple geometric figures, reproduce Koh's blocks designs, or separate visually embedded figures. When presented with a complex scene, she described only individual isolated features and was unable to give an overview of the interaction between components. Similarly, she lost track of the action when watching a movie when too many characters appeared in a scene. She no longer dreamed in visual images, but rather, described vague emotional experiences (e.g., 'suffocation') during sleep.

H.C. experienced topographical disorientation, and found it necessary to establish a system of markers to find her way around her own neighbourhood. Her ability to calculate was severely impaired, with loss of ability to perform arithmetical operations more complex than simple addition and subtraction. She showed no evidence of aphasia or disturbance of logical thought processes. An EEG and visual field examination by Goldmann perimetry (1° test object) were normal.

Interim history

Since her last evaluation by Adler in 1947, H.C. has remained in excellent general health. There have been no subsequent hospitalizations except for childbirth. She has remained physically and socially active, caring for her home and family, and retaining a particular fondness for ballroom dancing.

Through the years, however, she has frequently been frustrated by her inability to perform a wide variety of simple tasks. For example, she is unable to follow a recipe when cooking due to inability to discern demarcations on a measuring cup. She has difficulty finding her way in unfamiliar places and rarely reads, as this is no longer pleasurable. She feels that her deficits have prevented her from reaching her full potential in life and feels particularly deprived by her inability to work or to drive an automobile.

Until we located the patient in 1983, she had had no formal neurological reevaluation since leaving Adler's care. In the interim, she had consulted opticians and ophthalmologists in the hope that her visual difficulties could be corrected by proper refraction.

REEXAMINATION OF THE PATIENT

H.C., now 62-yrs-old, appeared younger than her stated age. General physical examination was unremarkable. She was socially appropriate and cooperative, with normal speech and affect. Recent and remote memory were excellent. Detailed cognitive testing is described below.

Corrected visual acuity was 20/30 bilaterally by Snellen chart. Visual fields were full to bedside testing with a 4 mm test object. She did not extinguish simultaneously-presented visual stimuli; she exhibited normal opticokinetic nystagmus. Fundoscopy by a consultant ophthalmologist was normal. Extraocular movements were full with smooth pursuits; the remainder of cranial nerve, motor and sensory examinations were normal.

Formal visual field testing by both tangent screen and Goldmann perimetry showed normal peripheral fields, but revealed an unexpected scotoma, which was homonymous, and which subtended the midline of the lower visual field (*see* fig. 1), encroaching on the central 10° of vision inferiorly. This visual field defect had not been detected on the patient's prior evaluations. Also, when a test object was moved along the inferior vertical meridian from the periphery, it was promptly detected; when the same object was moved along this meridian from the scotoma toward the periphery, it was not detected anywhere along its trajectory. (This discrepancy between the ability to detect centrifugal versus centripetal motion has been described in another case of carbon monoxide-induced visual agnosia (Campion, 1984), although the mechanism remains unexplained.)

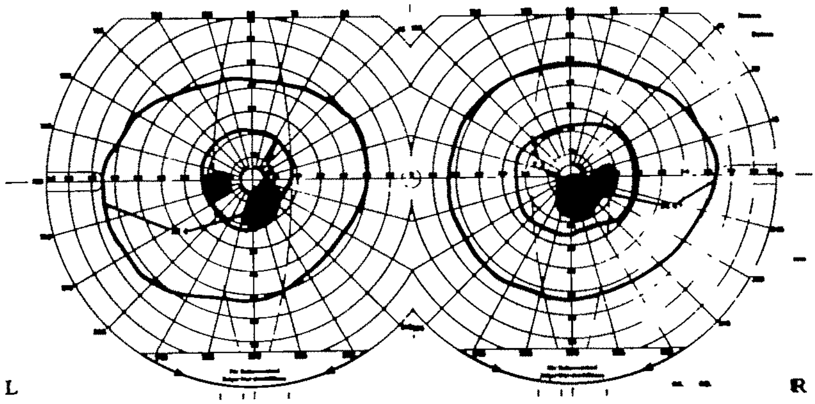

FIG. 1 Visual field examination by Goldmann perimetry.

A noncontrasted CT scan and T_1 weighted MRI (fig. 2A) showed bilateral occipital atrophy, but no other focal pathology. On T_2-weighted images (fig. 2B), patchy areas of increased signal were observed over the occipital convexity. These areas generally corresponded to widened occipital sulci on T_1-weighted images, although coexistent gliosis of the immediately adjacent subcortex could not be definitively excluded.

An EEG was normal. Pattern shift visual-evoked responses were normal in latency and waveform with full-field stimulation. Attempted quadrantic stimulation gave uninterpretable results.

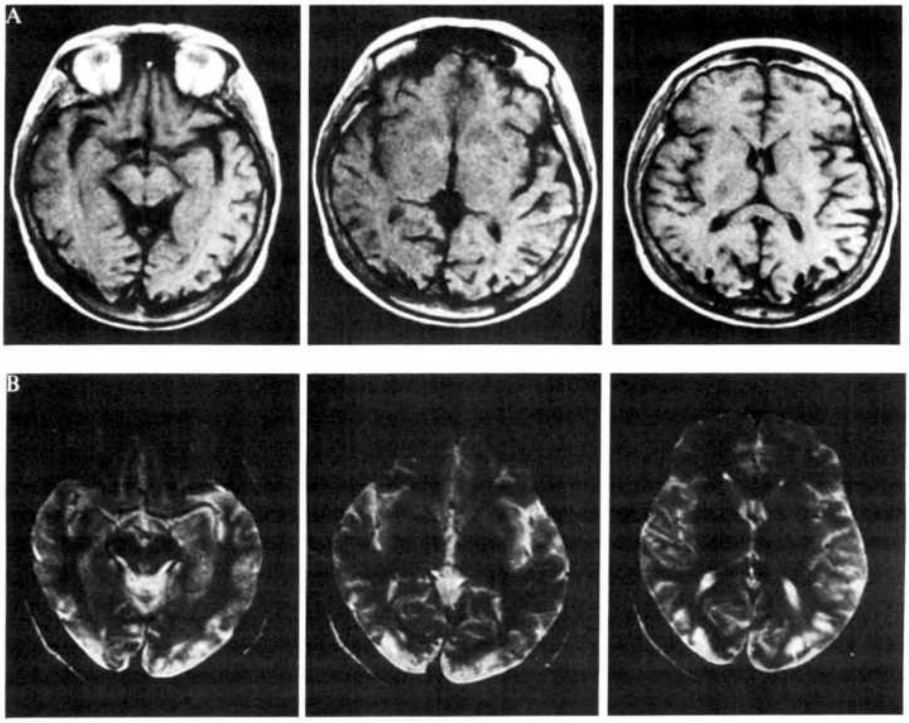

FIG. 2. MRI scan of the occipital region. A, T_1-weighted images (TE = 16, TR = 571); B, T_2-weighted images (TE = 85, TR = 2500). Left brain to viewer's right.

NEUROPSYCHOLOGICAL TESTING

Visual agnosia

H.C. had no difficulty identifying common objects presented visually. Formal testing, however, demonstrated a profound disorder of visual perception with characteristics similar to those described by Adler four decades earlier.

In general, when presented with visual materials, the patient appeared to perceive only small portions of the whole and from these small portions attempted to infer the overall scheme. She characteristically sought clues from the outlining contours from which she could construct or guess at the totality. This 'sum-of-parts' strategy often failed under certain testing conditions. For example, she was completely unable to identify visually-embedded Poppelreuter figures, in which multiple figural overlays defeat contour analysis. Similarly, she was unable to integrate visually the sectioned pictures of objects on the Hooper Visual Organization Test (1981). Only when these puzzle pieces contained defining information could she guess at the identity.

Several factors exacerbated her difficulties: small size, complex detail, and curved rather than straight lines. Her performance was near normal in selecting missing 'wallpaper patches' in the simpler designs of the Raven Standard Matrices (1960), but broke down with more complicated designs requiring pattern matching in more than one dimension.

Her use of contour analysis was well demonstrated by the manner in which she copied some of the simple geometric figures that Adler originally presented to her (*see* fig. 3A). Rather than copying the figure by its defining long lines, she perceived and copied small features of the contour, rendering a crude representation. This impairment extended to figures as simple as a hexagon (*see* fig. 3B).

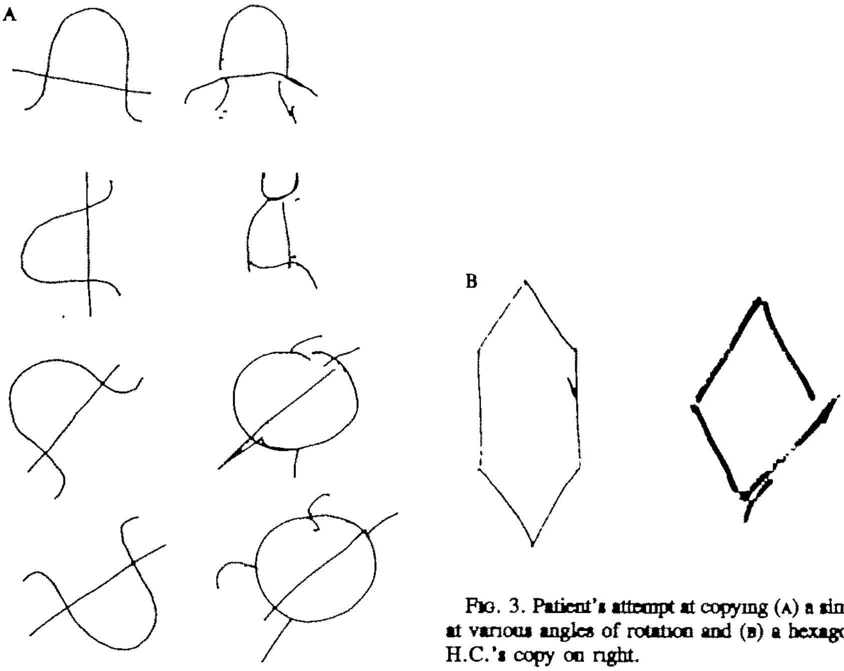

FIG. 3. Patient's attempt at copying (A) a simple geometric figure at various angles of rotation and (B) a hexagon. Stimulus on left, H.C.'s copy on right.

She demonstrated impaired constructions in replicating Kohs block designs, which she did slowly and with poor strategy. She was able to reproduce items 1–5 of the Block Design Subtest of the WAIS-R (Wechsler, 1981), but designs of the 9 block level were quite beyond her capabilities.

Additional visuospatial deficits included some degree of inattention to the right side of space when bisecting lines or attempting to locate the centre of a square. She showed poor topographical orientation and could not properly orientate to compass directions.

Colour naming and matching tasks were performed normally; she was able to identify colours presented to each hemifield without any sign of hemiachromatopsia.

Reading

The patient's alexia was essentially unchanged from that detailed by Adler in 1950. Although she is a high school graduate and formerly an avid reader, she now avoids reading as it has become so arduous. Formal assessment with the Durrell Analysis of Reading Difficulty (1955), Gilmore Oral Reading Test (1968) and the Woodcock Word Identification Test (1973) indicated that she read at approximately a third grade level of overall achievement.

At the single word level, she regularly misidentified individual letters, particularly in the interiors of words. For example, she read *front* as *first*, *soapy* as *sorry*, *farm* as *from*, and *smolder* as *smaller*. At the same time she was able to read and comprehend much more sophisticated words such as *jeopardize*, *physician*, and *sapphire*, although her overall abilities at this level were quite abnormal. Occasionally when she experienced difficulty discerning a word, she used her right index finger to trace one or more letters; this technique appeared to improve her performance.

At the sentence and paragraphic levels of reading, these letter and word misidentifications interfered with oral and silent decoding and comprehension.

Her difficulties were exacerbated by small print size, closely spaced lines, and highly stylized typeface. For example, she read the title of *The New York Times Magazine* as a 'Chinese Magazine' due to the highly stylized curvature of the letter *T*. Later, she identified the word *York*, but not the word *New*, which was superimposed on the boundary of light and dark of the cover photograph, suggesting difficulty in separating figure from ground.

Handwritten materials were particularly troublesome and she could not read a passage in her own handwriting shortly after writing it. Oral spelling, however, remained normal.

Writing

In her 5 yr follow-up study, Adler (1950) documented a deterioration in the patient's handwriting, with new distortions in letter formation, most notably the addition of extra loops to the letters *n, m, u,* and *y*. Adler speculated that this deterioration was the result of faulty visual feedback-correction, which ultimately altered internal percepts of letter formation.

Forty years later the patient's handwriting appeared to have stabilized at the 1947 level (fig. 4), with no further deterioration, but with persistence of the previously noted distortions.

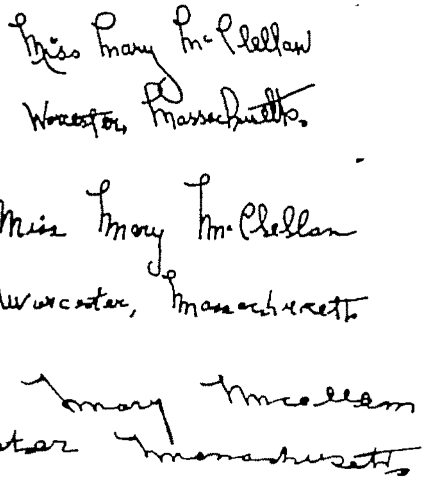
Miss Mary McClellan
Worcester, Massachusetts.
Miss Mary McClellan
Worcester, Massachusetts

FIG. 4. Writing to dictation. preinjury (*top*); 5 yrs after injury (after Adler, 1950) (*middle*), 40 yrs after injury (*bottom*).

Facial recognition

H.C. showed no difficulty in recognizing faces in a small group setting during examination, and readily recognized one of the examiners before he spoke on a chance meeting in the hospital elevator.

She showed poor performance, however, in recognizing photographs of well-known people, achieving only approximately 50% correct. Her performance improved with cuing (e.g., 'this is a world leader'), and with colour rather than black and white materials.

She often used contextual clues to help her guess at the identity. For example, when presented with a photograph of ex-President Carter shaking hands with a woman she stated, 'I don't recognize him too well . . . That's Carter. And that looks like his wife.'

Her piecemeal approach to analysing visual information was again apparent in her handling of caricatures and cartoons. For example, she correctly distinguished Bush from Reagan in a political cartoon stating, 'This one is Bush—he has a high forehead and not much hair'. She was unable to recognize a torso-only drawing of Mickey Mouse, but when told the correct identity said, 'Oh yes, I can see his eyes', pointing to two large buttons on his shorts.

Her ability to recognize faces markedly deteriorated when many people appeared in a photograph. Even with real faces she reported difficulty picking familiar faces out of a crowd. She stated, 'I never leave my seat on a plane trip because I'm afraid that I won't be able to find my husband again. You know how all people on a plane look the same . . . and they dress the same'.

Visual imagery

Disturbance of visual imagery was suggested by her poor performance on a number of tasks. Her free drawings were crude and similar in quality to efforts made in 1943 (fig. 5). The distortions in letter formation

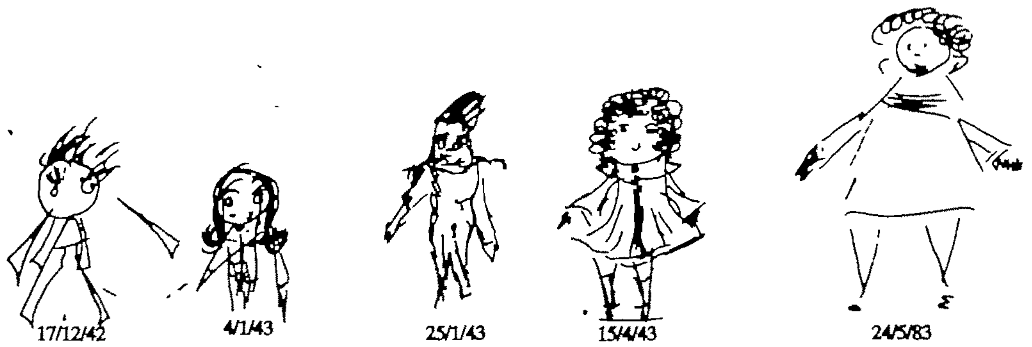

FIG. 5. Free drawing of a woman: performances shortly after injury (Adler, 1944) compared with present performance.

described above (*see* fig. 4) persisted when writing with eyes closed and when asked to describe verbally the structure of letters (e.g., 'an *m* has three loops').

When asked to imagine and describe an object, her response was to decompose the object into a few isolated component parts. She provided no description of the relationship or interaction of these parts, and gave no overview of the object as a whole. For example, she described a bicycle as 'a handle bar and two wheels' and an elephant as 'a big trunk . . . big feet . . . they are grey . . . they have little piercing eyes'. Topographical imagery was also impaired; she had difficulty giving directions for locations and routes familiar to her.

Adler documented the loss of visual content of dreams in her earlier reports. The persistence of this deficit could not be established on current examination as the patient denied recall of any dreams in recent years.

Language and intelligence

Absence of aphasia was established by testing of confrontation naming, speech production, repetition and verbal comprehension. The patient performed within normal range on the Token Test (De Renzi and Vignolo, 1962) and the Boston Diagnostic Aphasia Examination (Goodglass and Kaplan, 1972). Performance on the Revised Boston Naming Test (Kaplan *et al.*, 1978) was compromised by the patient's visual agnosia, but showed normal functional description naming and tactile identification naming.

Formal measurement of intelligence was limited to verbal testing to avoid errors introduced by her visual perceptual difficulties. Her Verbal Intelligence Quotient on the Wechsler Adult Intelligence Sale (WAIS-R) was within average range. The Wechsler Memory Scale (1945) demonstrated that immediate and delayed recall, and verbal new learning abilities were normal for her intelligence level.

DISCUSSION

Visual agnosia may be defined as the failure to recognize visually presented materials despite adequate visual acuity, visual fields, visual scanning, language function, and general mental ability (Alexander and Albert, 1983). In the strictest view, the deficit must be modality specific in that recognition of environmental stimuli via other sensory channels (auditory, tactile, olfactory) remains unimpaired.

As reported cases that conform to this definition are exceedingly rare, and differ so widely in localization, aetiology, associated neurological deficits and specific type of visual processing disturbance, a unifying theory of visual agnosia has not yet been achieved.

Lissauer (1890), on theoretical grounds, divided visual agnosia into associative and apperceptive varieties. Associative agnosics retain the ability to copy, match, or in some way indicate 'primary reception' of visual stimuli. Apperceptive agnosics fail at an earlier level of visual processing in that they are unable to demonstrate even primary reception. This classification has been criticized as over-simplistic and is unable to account for cases with mixed associative-apperceptive features (Kertesz, 1979; Riddoch and Humphreys, 1987). Nevertheless, the Lissauer classification continues to serve as a clinically useful starting point for analysing visual agnosias as the two variants appear to differ in localization and neuropsychological mechanism.

The associative form has been more thoroughly studied and is generally accepted to result from functional disconnection of visual centres from semantic stores ('visuolimbic disconnection', Alexander and Albert, 1983) due to lesions in connecting white matter pathways. Pathologically this disconnection arises from bilateral occipital injuries or, in a fashion analogous to the Dejerine (1892)-Geschwind (1965) hypothesis for pure alexia, can arise from unilateral dominant occipital injury with involvement of the corpus callosum (Bauer and Rubens, 1985).

The mechanism of apperceptive forms of visual agnosia remain less completely understood. Visual agnosia with apperceptive features has been described in cases of closed head trauma (Goldstein and Gelb, 1918), mercury poisoning (Landis *et al.*, 1982), and bilateral occipital infarctions (Riddoch and Humphreys, 1987), although, as will be discussed, it occurs most commonly and in purest form as the result of carbon monoxide toxicity.

Adler's report on her patient with visual agnosia in 1944 was an important milestone in the elucidation of the neuropsychological features of apperceptive visual agnosia, and has been acknowledged by subsequent investigators. Kurt Goldstein (1948) referred to Adler's work prominently in his exposition on disorders of *gestalt* formation; Benson and Geschwind (1969) cited this work in their review of alexia without agraphia and commented on the unusual deterioration of writing exhibited by this patient; Vincent *et al.* (1977) alluded to this case as a rare example of alexia without agraphia occurring without hemianopia or colour naming deficit; Ajax (1967) noted Adler's work as one of the few reports to document long-term prognosis in alexia; recent major review articles on visual agnosia by Alexander and Albert (1983) and Bauer and Rubens (1985) include reference to Adler's patient as a classic example of visual agnosia of the apperceptive type.

Observation of Adler's patient over a 40 yr period reveals that she evolved from a state of global visual impairment to a specific syndrome in which recognition of *form* is selectively impaired. She fails to recognize a shape as simple as a hexagon (*see* fig. 3) and yet readily recognizes real objects where size and colour clues help compensate for defective perception of form. Analysis of her performance on a wide variety of visual tasks indicates that she approaches complex visual material in a piecemeal fashion. She appears to reduce complex wholes into more manageable small components, but fails to recognize even these elemental components and thus does not succeed in reintegrating the parts into a coherent whole.

Many of the other features of her visuospatial disorder may, in fact, be consequences of this impaired recognition of form. These include simultanagnosia, that is, the tendency to focus attention of a small portion of the visual scene with failure to achieve an overview, inability to separate figure from ground, and impaired constructions.

The major functional consequences of her visual agnosia have been alexia and prosopagnosia. Although she has become quite adept at using compensatory strategies in reading and facial recognition, these strategies break down when she is faced with nonstandardized materials (e.g., stylized typeface) or with masking by a complex background.

In addition to her visual agnosia for the external environment, H.C. demonstrates faulty visual imagery and loss of visual content of dreams. These disturbances of *internal* visual representations were first reported a century ago by Charcot and by Wilbrand (*see* Nielsen, 1946) and have been described in scattered case reports since (*see* review by Farah, 1984). In these reports, as in the present case, disorders of visual imagery have been almost invariably associated with disorders of visual perception. These findings suggest that the neural networks used for visual perception and those for visual imagery may share at least some common elements (Levine *et al.*, 1985).

'Primary' visual testing showed normal visual acuity, visual tracking, and peripheral fields. A small inferior paracentral scotoma (*see* fig. 1) and inability to detect centrifugal motion along the inferior vertical meridian were noted, but cannot account for her complex visuospatial disorder.

Carbon monoxide-induced visual agnosia

With the exception of a few isolated cases with diffuse bioccipital injuries, apperceptive visual agnosia has been reported almost exclusively as the result of carbon monoxide cerebral toxicity. Adler noted 3 previous cases of visual agnosia induced by carbon monoxide (Schilder and Isakower, 1928; Solomon, 1932; von Hagen, 1941), and subsequently several more have been reported (Garland and Pearce, 1967; Benson and Greenberg, 1969; Abadi *et al.* 1981; Alexander and Albert, 1983; Campion, 1984), including the suggestive case of Cobb and Lindemann (1943) involving another victim of the Cocoanut Grove nightclub disaster.

These cases demonstrate a striking similarity. In general, after carbon monoxide exposure sufficient to induce loss of consciousness, the patient regains consciousness, is transiently in a confusional state, and ultimately becomes lucid. Memory disturbances and extrapyramidal signs are variably seen, and are consistent with the well-known toxicity of carbon monoxide on the hippocampus and basal ganglia (Lapresle and Fardeau, 1967; Ginsberg, 1980; Brierley and Graham, 1984). Initially the patient is cortically blind, then clears to a state of impaired visual perception with intact visual fields (or minor field defects) and normal visual acuity. The patient is unable to name accurately, copy or match visual stimuli (apperceptive visual agnosia). Frequently associated is the distortion of internal visual imagery and loss of visual content of dreams, the Charcot-Wilbrand syndrome (Nielsen, 1946). Some improvement may be seen early in the course, with nearly complete recovery reported in one case (Schilder and Isakower, 1928). More commonly, however, some degree of visual impairment persists and, as suggested by the present study, may become permanent.

In their detailed study of one such case, Benson and Greenberg (1969) found that their patient was able to detect subtle differences in luminescence, wavelength and area, but failed to distinguish differences in shape. They noted the similarity between their case and Adler's and suggested that this was a unique syndrome which they proposed be called *visual form agnosia*.

The anatomical locus of pathology in carbon monoxide-induced visual agnosia is unknown; there have been no postmortem studies. Benson and Greenberg proposed an *intracortical* localization, due either to laminar necrosis or patchy 'salt and pepper' cortical infarctions of the primary visual cortex, both of which are known consequences of carbon monoxide toxicity. This localization is appealing from a theoretical point of view as it places the lesion at an early stage of visual processing. On the other hand, carbon monoxide characteristically causes *subcortical* white matter degeneration, with occipital and splenial fibres frequently affected (Hsü and Ch'eng, 1938; Schwedenberg, 1959; Lacey, 1981). Subcortical localization would parallel the accepted pathogenetic mechanisms of other forms of disturbed visual recognition (associative visual agnosia and pure alexia), and would account for the relative specificity of carbon monoxide for this syndrome, as white matter degeneration is less frequently observed in hypoxic brain injury of other aetiologies.

In the absence of pathologically studied material, attempts have been made to distinguish between these proposed localizations by in vivo techniques: detailed visual field examination, neuroimaging studies, and visual-evoked potentials. Campion (1984) performed detailed mapping of the central visual field of a patient with carbon monoxide-induced visual agnosia and demonstrated multiple minute scotomata. He suggested that these arose due to patchy cortical infarctions, although scattered subcortical lesions could produce a similar clinical picture. Indeed, bilateral subcalcarine infarctions were visualized by CT scanning in Campion's patient. No such lesions, however, were evident on computerized tomography of our patient or a similar patient reported by Alexander and Albert (1983). MRI scanning performed in our patient demonstrated focal occipital atrophy bilaterally. There were no discrete lesions visible in the deep white matter although injury to immediately subcortical white matter could not be excluded with certainty.

Pattern reversal visual-evoked responses were absent in the patient of Alexander and Albert, which they felt was indicative of extensive damage to occipital cortex. Normal responses were elicited in our patient. These normal responses, however, do not exclude laminar necrosis of the occipital cortex as recent studies in primates suggest generation of most components of the VEP in lamina IV (Kraut *et al.*, 1985), which is highly resistant to laminar necrosis. Thus the question of localization of carbon monoxide-induced visual agnosia to cortical versus subcortical structures remains unresolved and may ultimately require pathological examination for resolution.

In summary, carbon monoxide toxicity can induce a visual agnosia with apperceptive features, seldom seen with other types of cerebral injury. This syndrome occurs after recovery from cortical blindness and is a characterized misperception of elemental shape and form, alexia, and impairment of visual imagery. Pathology is localized to bilateral occipital lesions. Long-term prognosis for recovery of visual function is poor.

ACKNOWLEDGEMENTS

The authors wish to thank Dr Joseph Rizzo, Department of Ophthalmology, Boston City Hospital, for performing detailed ophthalmological and visual field testing. We especially wish to convey our gratitude to Dr Alexandra Adler whose publications inspired our efforts and who provided invaluable assistance in helping us to locate the patient.

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(Received March 7, 1989. Revised April 27, 1990. Accepted May 15, 1990)

Brain (1991), **114**, 801–810

PATTERNS OF BLOOD-BRAIN BARRIER BREAKDOWN IN INFLAMMATORY DEMYELINATION

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SUMMARY

The evolution of the changes in the blood-brain barrier (BBB) in chronic relapsing experimental allergic encephalomyelitis (CREAE), a model of immune-mediated demyelination, has been studied by magnetic resonance imaging (MRI); gadolinium-DTPA (Gd-DTPA) was used to detect BBB breakdown by both quantitative and qualitative techniques. Animals with acute EAE were examined for comparison. In animals with CREAE an approximately linear relationship was found between the mean number of lesions enhancing with Gd-DTPA seen per MRI slice and the severity of clinical disability at relapse. In addition, a direct relationship was seen between the duration of clinical relapse and the duration of enhancement with Gd-DTPA for lesions associated with the relapse. Lesions studied in animals having entered a progressive phase of disease showed the most sustained BBB breakdown. These observations suggest that BBB breakdown is important in the development of clinical signs in inflammatory demyelination. In CREAE, areas of focal enhancement with Gd-DTPA could usually be clearly defined at a time of clinical relapse. In slices free of focal lesions, no abnormal Gd-DTPA leakage could be detected using a quantitative method. In contrast, in acute EAE no focal lesions were visible, but significant leakage was detected by measurement. No change was found in T_2 relaxation times in CREAE or acute EAE.

The pattern of BBB breakdown in inflammatory demyelination evolves from a diffuse shortlived disturbance in acute EAE to a more focal and prolonged breakdown in animals with chronic relapsing and progressive disease. The broad similarities in the pattern of BBB breakdown seen in CREAE and multiple sclerosis support the hypothesis that the initial vascular changes in the human disease are due to inflammation which could be mediated immunologically.

INTRODUCTION

Enhancement with gadolinium-DTPA (Gd-DTPA) is a feature of new lesions seen with magnetic resonance imaging (MRI) in patients with active multiple sclerosis (MS) (Grossman *et al.*, 1986; Gonzalez-Scarano *et al.*, 1987; Kappos *et al.*, 1988; Miller *et al.*, 1988*a*; Kermode *et al.*, 1990*a*). An area of enhancement following Gd-DTPA injection may be seen before the appearance of a lesion on the unenhanced T_2-weighted image (Kermode *et al.*, 1990*b*); the importance of blood-brain barrier (BBB) breakdown in the early stages of evolution of the MS plaque seems without doubt, but the relationship of barrier breakdown to the observed clinical disability at relapse, and the changes in the BBB with progression of the disease, are unclear.

There are important similarities between chronic relapsing experimental allergic encephalomyelitis (CREAE) in guinea-pigs and MS (Lassmann, 1983). We have

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previously shown that enhancement with Gd-DTPA in CREAE corresponds with areas of BBB breakdown as judged by traditional barrier markers. Enhancement was related to perivascular inflammation (which was often confluent) and demyelination histologically in CREAE, and to inflammation alone in acute EAE (Hawkins *et al.*, 1990).

An important question raised by this study was whether there is a relationship between the timing and degree of BBB breakdown and the development and severity of clinical signs in inflammatory demyelination in relapsing disease. We have therefore sought to determine whether there is a changing pattern of BBB breakdown which is detectable by MRI during the evolution of disease, from acute and diffuse inflammatory EAE through to chronic relapsing and finally progressive demyelinating disease. Gd-DTPA-enhanced MRI, which permits serial measurements of BBB breakdown in vivo, has allowed this question to be answered without the restrictions imposed by the use of traditional BBB markers in terminal experiments (Kristensson and Wiśniewski, 1977; Grundke-Iqbal *et al.*, 1980).

METHODS

CREAE was induced in 15 juvenile (17–22-day-old) strain 13 guinea-pigs by two 0.1 ml subcutaneous injections into the dorsa of the hindfeet. The inoculum contained 50% (w/v) homogenate of guinea-pig spinal cord in phosphate-buffered saline (PBS) added to an equal volume of complete Freund's adjuvant (CFA), containing 10 mg/ml Mycobacterium tuberculosis (Difco: H37Ra). Acute EAE was induced in 5 adult Hartley guinea-pigs by the same method. Control guinea-pigs (5 strain 13 animals) received an emulsion of 1:1 (w/v) PBS/CFA only, and were studied at the same time after injection as were the CREAE animals (between 2 and 6 months after inoculation). Animals were weighed and clinical assessment recorded three times per week in CREAE and daily in acute EAE, using the following scale: stage 0.5 = loss of righting reflex; 1 = minimal weakness/unsteadiness of hindlimbs; 1.5 = unable to weight bear on one or other hindlimb; 2 = unable to weight bear on either hindlimb; 2.5 = complete paralysis of one hindlimb; 3 = complete paralysis of both hindlimbs; 3.5 = +partial weakness of forelimbs; 4 = +complete paralysis of forelimbs. Five animals with CREAE showing a relapsing and remitting course were selected for study, and were followed through to a progressive phase of disease if this occurred.

Gd-DTPA (Schering AG) was injected i.v. in a dose of 0.5 mmol/kg. MRI was performed using a 0.5 Tesla machine (Picker International). The lumbar spinal cord was routinely scanned in transverse (axial) plane with 2 mm slice thickness and 10 cm field of view (256 values of phase encoding gradient with pixel size 0.4 mm×0.4 mm). Specially built surface receiver coils were used to optimize the signal-to-noise ratio.

Anaesthesia for MRI was induced by a Hypnorm/Hypnovel (Janssen, Kent, UK) mixture (1 part fentanyl-fluanisone 0.32 mg/ml–10 mg/ml, 1 part midazolam 5 mg/ml and 2 parts sterile water) in a dose of 8 ml/kg by intraperitoneal route. An additional 4 ml/kg after 1 h prolonged anaesthesia for up to 4 h. The animals were wrapped in cotton wool during the experiment to maintain a core (rectal) temperature between 36° and 37.5° C.

To determine the duration of Gd-DTPA enhancement for individual lesions, the 5 CREAE animals with relapsing and remitting disease were scanned serially twice per wk for between 4 and 12 wks; 3 animals entered a progressive phase of disease while being studied.

A Carr-Purcell-Meiboom-Gill (CPMG) multiecho sequence (ME 2000/40-640, single slice, 2 repetitions over 17 min) was used for imaging and measurement of T_2 relaxation time from the magnetization decay curve. Values of mean signal intensity for each echo time were entered into a least squares fit program to determine the best fit for a monoexponential decay and, if present, a biexponential magnetization decay. Values were taken from the CPMG images where the signal-to-noise ratio remained more than 2 (which was usually the case for at least 8 echoes). It has been shown that with due care relaxation times measured using our 0.5T imager are accurate and similar to values obtained using the imager as a spectrometer (Johnson *et al.*, 1987).

For detection of areas of focal enhancement with Gd-DTPA and quantitative assessment of Gd-DTPA leakage, an inversion recovery sequence was used, before and after injection of contrast agent (IR 560/300/40, 2 slices, 4 repetitions over 10 min). This was repeated serially for 1 h after injection with the signal attenuation set to a constant value and using magnitude image reconstruction. Using this sequence the measured MRI signal increases linearly with the T_1 relaxation rate (i.e., $1/T_1$), and for the relaxation times being measured the signal is always positive. The increase in T_1 relaxation rate is proportional to the mean Gd-DTPA concentration which has leaked into the tissue. The maximum signal following contrast injection is expressed as a fractional increment relative to the mean signal from the same MRI slice prior to injection. The measured increment is approximately proportional to Gd-DTPA leakage.

Gd-DTPA leakage and T_2 relaxation time were measured in the normal-appearing cord of CREAE and control animals under study. For both measurements, sampling was a mean value of the whole spinal cord in transverse section, obtained using a program supplied by the manufacturer (Picker), taking care to exclude the meninges and CSF space from the calculation. For comparison, 5 Hartley guinea-pigs with acute EAE were studied in the same way with images and calculation for Gd-DTPA-enhancement and CPMG sequences for T_2 measurement. Measurements were taken before inoculation for control values, and at 7, 10 and 14 days after inoculation; and at 21 days in 3 animals which remained alive. For measurements of Gd-DTPA leakage and T_2 relaxation times: in CREAE, acute EAE and control animals, sampling was of the lumbar cord from 2 adjacent slices, and each mean value for the transverse slice being studied included either 16 or 20 pixels of the image. Care was taken in serial studies to ensure that the same MRI slices of the spinal cord were measured on each occasion by using oblique pilot imaging facilities supplied by the manufacturer. The chosen MRI slices were designated by their alignment with adjacent vertebral bodies on the sagittal pilot scan.

Images were assessed by C.P.H. and reviewed blindly by E.P.G.H. du B. For the duration of lesion enhancement, only areas of focal enhancement with Gd-DTPA seen to be definite by both observers were included in the study. For quantitative measurements, assuming an approximately normal distribution of relaxation time values, the results were analysed using Student's t test; an unpaired test for results from CREAE, and a paired test for acute EAE (a paired test was used for the latter group because it was possible to measure preinoculation control values in these animals before induction of EAE).

RESULTS

In control animals there were no areas of focal abnormality seen in the spinal cord on Gd-DTPA-enhanced or unenhanced scans. In CREAE animals with a relapsing and remitting course, the mean interval between clinical relapses was approximately 3 wks (range 1–6 wks). The development of areas of focal enhancement with Gd-DTPA seen on the inversion recovery scans corresponded broadly with episodes of clinical relapse (for over 90% of the Gd-DTPA-enhancing lesions observed). It was noticeable, however, that 25% of enhancing lesions were visible before (less than 5 days) the onset of new clinical signs at the time of clinical relapse, and 30% of lesions were not visible until after the onset of clinical relapse (enhancement appearing within 5 days after the onset of new clinical signs). With clinical recovery, 80% of the lesions associated with the relapse had ceased to enhance; the remaining 20% continued to enhance for up to 5 days after this. The mean number of Gd-DTPA-enhancing lesions per slice showed an approximately linear relationship to the severity of clinical disability at relapse ($r = 0.71$; fig. 1).

Most lesions studied in chronic relapsing and remitting animals were seen to enhance for a relatively short period of time (less than 15 days, and many between 5 and 10 days) (fig. 2). It was observed that frequently (30% of enhancing lesions) there was recurrence of enhancement with Gd-DTPA in areas of the cord where there had previously been enhancement which had ceased, and in most of these cases recurrence of enhancement was associated with a further clinical relapse. A direct relationship was

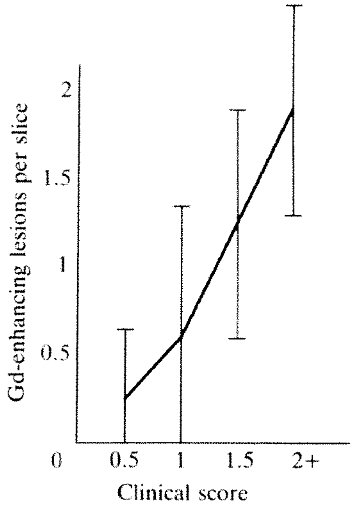

FIG. 1. An approximately linear relationship is demonstrated between the mean number of Gd-DTPA-enhancing lesions detected per slice during the evolution and recovery of clinical relapse and the grading of the severity of clinical disability. Horizontal bars represent 1 SD above and below the mean. The number of values from which the results of enhancing lesions/slice were calculated is as follows: for severity of disability graded as 0.5, n = 7; for grade 1, n = 5; for grade 1.5, n = 10; and for grade 2+, n = 4.

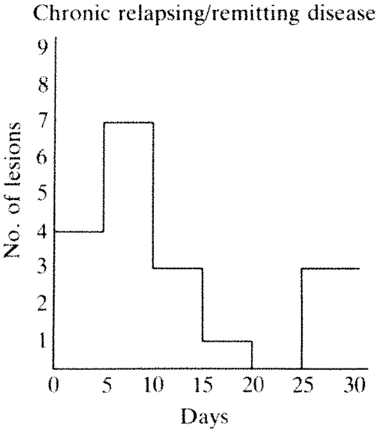

FIG. 2. Duration of enhancement with Gd-DTPA for individual lesions is shown in chronic relapsing and remitting disease (5 animals studied). Horizontal axis represents duration of enhancement in days determined by serial scanning of the animals with Gd-DTPA injection on each occasion.

seen between the duration of the clinical relapse and the duration of enhancement with Gd-DTPA for lesions associated with the clinical relapse (fig. 3). Lesions observed in animals having entered a progressive phase of disease showed the most sustained enhancement, 3 out of 5 continuing to enhance for more than 3 wks.

Gd-DTPA enhancement was seen to be focal in CREAE, affecting the dorsolateral as frequently as the lateral and ventrolateral cord in the relapsing and remitting animals as well as in those animals that entered a progressive phase of disease; there was no measurable leakage (as determined by the quantitative method) in the normal appearing cord compared with control animals (Table 1). This was in contrast to acute EAE when no definite focal lesions could be identified, but diffuse Gd-DTPA leakage was detected by measurement, being maximal between 7 and 14 days after inoculation (Table 2).

When these measurements were considered in relation to clinical signs in acute EAE (excluding weight loss), there was a mean increment in signal (after Gd-DTPA injection)

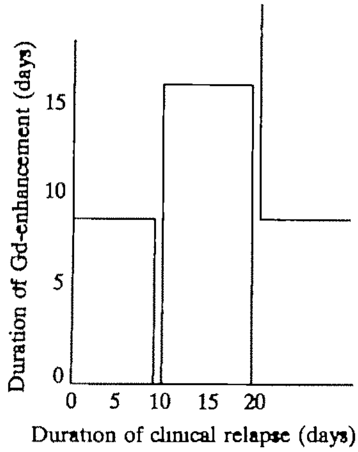

FIG 3 A direct relationship is observed between the duration of clinical relapse in chronic relapsing and remitting disease and the duration of enhancement with Gd-DTPA for lesions associated with the clinical relapse. Duration of enhancement was established by serial scanning (fig 2). Duration of clinical relapse is represented by 3 ranges on the horizontal axis (less than 10 days, between 10 and 20 days or more than 20 days), and the observed duration of Gd-DTPA enhancement established for individual lesions in CREAE is similarly represented on the vertical axis (less than 8 days, less than 16 days, and more than 8 days; the number of lesions in each category was 4, 10 and 4, respectively).

of 0.32 between 3 and 7 days before the onset of clinical signs. This was statistically highly significant ($P < 0.005$) compared with control values before inoculation. The mean difference in increment, between this value for Gd-DTPA leakage in acute EAE before the onset of clinical signs and preinoculation values, was 0.23, with a 95% confidence interval between 0.15 and 0.31.

Maximum enhancement following Gd-DTPA injection always occurred within 60 min, and in over 70% of individual studies the measured signal enhancement following injection had returned to preinjection values within the 60 min; this applied to visible focal enhancement in CREAE and to measurements of diffuse enhancement in acute EAE. In the case of focal enhancement in CREAE, approximately 20% of the lesions showed maximum enhancement on the first inversion recovery scan following Gd-DTPA injection (within 10 min of injection); 40% of the lesions did so after 20 min of scanning, and the remaining 40% after 30 and 40 min. In contrast, for measurements of leakage in acute EAE, 50% of MRI slices showed maximum values within the first 10 min, 20%, after 20 min, and the remaining 30%, after 30 min.

Control animals (both CFA-injected, which were control animals to study CREAE, and noninjected animals for preinoculation control values before induction of acute EAE)

TABLE 1 CHRONIC RELAPSING EAE. QUANTITATIVE RESULTS OF Gd-DTPA LEAKAGE AND T_2 RELAXATION TIME

	Gd-DTPA leakage (increment in signal)	*T_2 relaxation time (ms)*
CREAE		
Normal-appearing cord (no areas of focal enhancement)	0 11 (0 09)	92 (10)
Focal Gd-DTPA-enhancing lesions (bilateral)	0.17 (0.06)*	96 (9)
Control	0 07 (0.08)	96 (10)

Figures in brackets represent 1 SD * Significantly different from control values by unpaired Student's t test ($P < 0\ 05$)

TABLE 2. ACUTE EAE: QUANTITATIVE RESULTS OF Gd-DTPA LEAKAGE AND T_2 RELAXATION TIME

	Gd-DTPA leakage (increment in signal)	*T_2 relaxation time (ms)*
Preinoculation	0.09 (0.10)	98 (9)
Postinoculation		
7 days	0.29 (0.12)*	99 (4)
10 days	0.23 (0.09)*	93 (8)
14 days	0.37 (0.20)*	92 (8)
21 days	0.09 (0.07)	96 (2)
Related to the onset of clinical signs (= day 0)		
−7 to −3 days	0.32 (0.14)*	97 (4)
−2 to +2 days	0.26 (0.12)*	93 (10)

Figures in brackets represent 1 SD. * Significantly different from preinoculation (control) values by paired Student's t test ($P < 0.005$).

showed a minor but measurable enhancement with Gd-DTPA, the mean increments in MRI signal being 0.07 and 0.09 for the two groups (Tables 1, 2). In these animals maximum values for enhancement occurred within 10 min of Gd-DTPA injection in only 30% of the MRI slices examined, and not until after 20 min of scanning for 50% of the measurements and 30 min for the remaining 20%. This suggests that enhancement with Gd-DTPA in control animals may reflect a low grade and relatively slow passage of the small molecule Gd-DTPA (molecular weight 550 Da) across the normal BBB, rather than enhancement being due to an intravascular effect of the intravenous injection of Gd-DTPA, when maximum enhancement in the majority of slices would be expected to occur very soon after injection (within 10 min).

Inversion recovery scans showed no areas of focal abnormality on any occasion before the injection of Gd-DTPA in the serial studies of Gd-DTPA-enhancement (and leakage) in CREAE or acute EAE. Multiecho sequences (unenhanced) showed no definite lesions in CREAE or acute EAE. T_2 relaxation time measurements from normal-appearing cord showed no significant change in chronic or acute EAE when compared with control values (Tables 1, 2). All T_2 magnetization decay curves were found to fit a monoexponential magnetization decay, and values to fit a biexponential curve could not be generated from the measurements obtained in this study using our least squares program.

DISCUSSION

Animals with chronic relapsing and remitting EAE showed areas of focal Gd-DTPA enhancement, and many lesions enhanced for a period of less than 15 days (many between 5 and10 days). There was a direct relationship between the duration of clinical relapse and the duration of enhancement with Gd-DTPA for lesions associated with the relapse. In animals having entered a progressive phase of disease some lesions were still enhancing after 3 wks study. These observations complement those previously reported in animals with well established progressive disease, when enhancement for individual lesions often continued for more than 5 wks. The duration of enhancement for focal areas in acute

EAE, however, was of shorter duration (always less than 5 days) (Hawkins *et al.*, 1990). The present results suggest that the development of progressive disease in CREAE is related to a more prolonged or continuing breakdown of the BBB.

There are important clinical and histological similarities between CREAE in guinea-pigs and MS (Wiśniewski and Keith, 1977; Lassmann, 1983), and our observations raise the possibility that actively progressive disease in MS, at least of the secondary progressive type (Thompson *et al.*, 1989), may be related to a prolonged or continuing and repeated breakdown of the BBB. A study to determine whether this is so is in progress.

The different patterns of Gd-DTPA-enhancement in the different groups of EAE were found to reflect differences in measurements of Gd-DTPA leakage in areas of the spinal cord without visible focal enhancement, the cord appearing normal at MRI. In the cord free of visible focal lesions in acute EAE, diffuse Gd-DTPA leakage could be measured at a stage when maximal barrier breakdown would be expected to occur in the guinea-pig model, from studies using conventional barrier markers (Vulpe *et al.*, 1960; Holländer and Mehraein, 1965; Cutler *et al.*, 1967; Leibowitz and Kennedy, 1972). This is usually at a time when there is diffuse perivascular inflammation histologically. In contrast, in CREAE the normal-appearing cord showed no measurable leakage of Gd-DTPA when compared with control values and enhancement was detectable only as focal areas of BBB breakdown, reflecting areas of confluent inflammation and demyelination histologically (Kristensson and Wiśniewski, 1977; Lassmann, 1983; Hawkins *et al.*, 1990).

There was also evidence for different degrees of permeability of the disrupted BBB at different stages of EAE (acute EAE and CREAE). Maximum leakage of Gd-DTPA following individual injections in acute EAE was found to occur within 10 min of injection in half of the MRI slices studied, suggesting a rather high permeability breakdown of the BBB. In contrast, in CREAE maximum enhancement of focal lesions was observed usually at a later stage, occurring within 10 min of injection in only 20% of the lesions, and in 40% not until after 30 and 40 min of scanning. This would suggest a lower permeability breach of the barrier to the relatively small molecule, Gd-DTPA, for lesions in CREAE than in acute EAE.

In chronic relapsing and remitting disease there was a linear relationship between the number of Gd-DTPA-enhancing lesions per slice seen at a particular stage during the evolution of a clinical relapse and the grading of the severity of the clinical signs observed at that time. This is despite the timing of the first appearance of enhancement for individual lesions often not corresponding exactly with the onset of clinical relapse. This implies that the extent of BBB breakdown in the spinal cord is important in relation to the development of clinical signs in relapsing animals. How this relates to axonal function is uncertain. Oedema has been suggested to play a role (Paterson, 1976), but while this may well be true in regions of tight constriction where compressive effects may operate (e.g., the optic nerve in the optic canal; Miller *et al.*, 1988*b*), the frequent lack of symptoms in the face of extensive oedema surrounding a variety of focal cerebral lesions suggests that other factors appearing with a similar time course are more important. These might include complement-induced myelin damage (Scolding *et al.*, 1989) and inflammatory mediators such as the interleukins or tumour necrosis factor (Brosnan *et al.*, 1988). A study of their effects on conduction would be well worthwhile.

The T_2 relaxation time measurements showed no change in acute or chronic EAE compared with control values. This observation has previously been reported for acute EAE (induced by inoculation with CNS tissue) using in vitro measurements (Karlik *et al.*, 1986); no change in T_2 or T_1 was found in that study. It is probable that protein exudation, accompanying vascular inflammatory changes and BBB breakdown, is sufficient to maintain the T_2 values within the normal range, despite the expected increase in T_2 values which would accompany oedema (Karlik *et al.*, 1986; Hawkins *et al.*, 1990).

There is thus a gradual evolution of the pattern of enhancement with Gd-DTPA in CREAE which may be relevant to our understanding of the pathogenesis of inflammatory demyelination in MS. First, in acute EAE with predominantly diffuse perivascular inflammatory changes at histology there is diffuse leakage of Gd-DTPA which can be measured from changes in T_1 in the absence of visible focal enhancement. Next, with the development of chronic relapsing and remitting disease, areas of focal Gd-DTPA enhancement are frequently seen and correspond with the development of areas of focal and confluent inflammation with demyelination at histology. The duration of Gd-DTPA-enhancement for individual lesions in relapsing and remitting disease, however, is still relatively short (less than 15 days). Last, with the development of secondary progressive disease the duration of enhancement for individual lesions becomes more prolonged.

It is probable that recurrent immune-mediated attack on the small veins and venules in an area of the cord in CREAE accounts for the frequent recurrence of enhancement with Gd-DTPA in the same general area in chronic relapsing and remitting disease, although whether the renewed enhancement is from previously spared vessels within this area, previously damaged vessels, or both is at present uncertain. If, as seems likely, previously damaged vessels are vulnerable to further immune-mediated damage it is possible that recurring damage delays the repair process, resulting in the more prolonged BBB breakdown of the established lesions seen in progressive disease. This implies that an area of the cord, previously affected by inflammatory changes and BBB breakdown, is predisposed to further BBB breakdown with continuing immune attack. Active demyelination accompanies inflammation with BBB breakdown in CREAE, and thus prolonged barrier breakdown would be expected to result in a chronic demyelinated plaque (Lassmann, 1983).

It should be mentioned that the dose of Gd-DTPA used in this study (0.5 mmol/kg) to detect focal lesions in the spinal cord, is higher than that which would normally be used in clinical studies; the conventional dose currently used is 0.1 mmol/kg, or sometimes 0.2 mmol/kg. In the present study there were no areas of focal abnormality seen on inversion recovery scans on any occasion prior to serial Gd-DTPA injections, which might suggest that there is an important accumulation of contrast agent in an expanded extracellular space in the lesions. Thus it seems unlikely that such a mechanism is leading to error in our interpretation of serial enhancement with Gd-DTPA and the duration of barrier breakdown for individual lesions.

There is good evidence for immune deviation in MS (Calder *et al.*, 1989; Hafler and Weiner, 1989), and that a very early event in the development of a new lesion in relapsing and remitting and secondary progressive disease is a breakdown in the BBB (Thompson *et al.*, 1989). A comparison of duration of enhancement with Gd-DTPA in relapsing/remitting and progressive phases of MS has yet to be made, but the broad similarities between its duration and pattern in CREAE and MS (Miller *et al.*, 1988*a*), and the similarity in the morphology of enhancement in MS and the distribution of inflammation at postmortem (Kermode *et al.*, 1990*a*) provide support for the hypothesis that the initial vascular changes in these forms of MS are due to inflammation which could be mediated immunologically. Recurrence of enhancement in previously enhancing areas occurs in the human as in the experimental disease (A. G. Kermode *et al.*, unpublished results). The development of focal lesions in MS might therefore relate to a predisposition of previously affected vessels to further immune attack and recurring BBB breakdown with inflammation would lead to demyelination, as in CREAE.

Recent work has provided evidence for significant differences between primary and secondary progressive MS; in the former, visible MRI lesions are smaller, appear less frequently and very rarely show Gd-DTPA enhancement (Thompson *et al.*, 1989, 1990), The last observation cannot necessarily be taken to mean that inflammation is absent, as our observations on acute EAE show. Application of the quantitative method which in the latter provided evidence for diffuse Gd-DTPA leakage, which is associated with correspondingly diffuse inflammation histologically, should establish whether similar diffuse changes beyond the resolution of the scanner are present in this distinctive group of patients. It would also be interesting to apply the method to the normal-appearing white matter early in the course of MS to determine whether there are diffuse changes at this stage (as there are at postmortem; Allen *et al.*, 1981; Adams, 1988) which precede the development of persistent visible focal MRI lesions.

ACKNOWLEDGEMENTS

The Multiple Sclerosis NMR Research Group was established with a generous grant from the Multiple Sclerosis Society of Great Britain and Northern Ireland and additional support is provided by the Medical Research Council. We are grateful to Picker International for their continuing research support, and to Schering AG for supplying the Gd-DTPA used in this study.

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(*Received December 8, 1989. Revised May 1, 1990. Accepted May 16, 1990*)

Brain (1991), 114, 811–824

CEREBRAL AKINETOPSIA (VISUAL MOTION BLINDNESS)

A REVIEW

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SUMMARY

Cerebral akinetopsia is a syndrome in which a patient loses specifically the ability to perceive visual motion following cortical lesions outside the striate cortex. There has been only one good case of akinetopsia in the published literature. Yet that case was immediately accepted by the neurological world. In this, cerebral akinetopsia differs markedly from cerebral achromatopsia, the evidence for which was strongly contested for the better part of a century (Zeki, 1990). This article complements the one on cerebral achromatopsia, traces the history of akinetopsia and enquires into why it was so much more readily acceptable than achromatopsia.

INTRODUCTION

In 1983, the world of neurology witnessed two surprises. The first was the publication of a paper by Zihl *et al.* describing a patient who became defective in her ability to see objects in motion following a bilateral cerebral vascular lesion in cortex outside the striate area. Apart from a mild anomic aphasia, the defect was specific for visual motion perception and was not accompanied by a scotoma. It was the first clinical description of cerebral motion blindness, a syndrome which will be referred to as *akinetopsia* in this review, to bring it into line terminologically with the much more common achromatopsia, or cerebral colour blindness. The second surprise was that, although a single case study, it was immediately accepted by the neurological and, more generally, by the neurobiological world, without a murmur of dissent. This was in marked contrast to the more turbulent history of achromatopsia, the evidence for which was strongly challenged. Here there was no MacKay (1888) to complain that 'the cases are very few in number', no Henschen (1910) to claim that his 'cortical retina' (the striate cortex) is also a retina for '(movement) impressions', no Critchley (1965) to write of '. . . a mere handful of instances of alleged (motion) agnosia, most of which are unconvincing'. Compared with the many, and conceptually seemingly powerful, objections raised against the notion that cerebral achromatopsia results from lesions in a visual centre outside the striate cortex (*see* Zeki, 1990, for a review), this acquiescence is surprising; it makes one want to learn more about the early literature regarding disturbances of visual motion and contrast it with the literature on achromatopsia.

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Fortunately, that literature is very brief compared with the literature on achromatopsia. It has made no impression at all in the world of neurology.

EARLY HISTORY

Akinetopsia can be defined as a defect in the perception of visual motion. Like achromatopsia, it may occur in conjunction with other defects or manifest itself as a remarkably specific syndrome, as in the patient of Zihl *et al.* (1983). One of the earliest clinical papers describing akinetopsia was that of Pötzl and Redlich (1911). Following bilateral injury to the occipital lobes, their patient could neither detect moving objects nor fixate them when they approached her quickly. The akinetopsia was linked to a 'defect of peripheral vision with retention of light perception and colour vision (and with) evidence of mental blindness and disturbance of spatial orientation'. In addition, the patient suffered from disturbances of orientation and of recognition as well as the inability to localize acoustic stimuli. There was no attempt to localize the lesion, or discuss its extent. The paper is not an important one in the clinical literature and the akinetopsia described there, as well as in the later paper of Goldstein and Gelb (1918), can be attributed to a more general disturbance following lesions which were not verified by postmortem examination and the extent of which therefore remains unknown. This indeed was the interpretation given by Jung (1949) who re-examined the patient of Goldstein and Gelb in 1942 and found that the patient was not deficient in the perception of shapes and movements. In the absence of postmortem material, Jung concluded that it was more likely that the patient had suffered from a chiasmal defect rather than a parieto-occipital lesion, as Goldstein and Gelb had believed. Jung stressed 'The importance of elaborated investigations in single cases of brain injury', a cautionary remark not dissimilar to that delivered much earlier by MacKay (1888) with respect to achromatopsia. MacKay had urged ' . . . the desirability of investigating cases of hemianopsia with more thoroughness and precision than is usually shown', an exhortation which did nothing to prevent him from accepting the syndrome of achromatopsia 11 years later, on the basis of a single patient whose 'light sense' could not be studied 'for want of proper means in the patient's house'! (MacKay and Dunlop, 1899).

Just as achromatopsia has its obverse, so does akinetopsia. The obverse of achromatopsia is the selective sparing of colour vision in cases of acute carbon monoxide poisoning, first reported by Wechsler (1933). The first suggestion that visual motion may be selectively spared was made by Riddoch in 1917. He had been studying patients blinded by gunshot wounds during the Great War. He had found that, in several, the extent of the scotoma was not the same when he had plotted the scotomatous field with stationary and with moving objects, the scotomatous field being commonly smaller when plotted with moving stimuli. The patients had difficulty in describing the characteristics of the moving objects, 'But they are quite sure that neither shape nor colour can be attributed to it, and that it can be detected in a field which is entirely blind to stationary objects'. Moreover, he had found not only 'That the recovery of vision for movement preceded that for the object' but also 'That the amount of recovery was greater for movement than for the object' and began in the peripheral field. These findings had led him to conclude that 'Movement may be recognized as a special visual perception', separate and in addition to the perceptions ' . . . of light, of form, and of colour'. Riddoch's evidence for a dissociation of motion vision was thus positive, in the sense that motion vision was relatively spared, while the remaining visual capacities were severely compromised. Here there are three similarities between the literature on colour vision and motion vision disturbances following cortical lesions. The first is that the literature shows that both can be selectively damaged but also selectively spared, or at least affected much less than the other submodalities of vision. The second is that the literature of both conditions leaves obscure the neural mechanisms responsible for the selective sparing; and the third is that neither literature describing the sparing of these attributes has made the slightest impression in the neurological world.

No sooner had Riddoch published his paper than his evidence was quickly dismissed by Holmes (1918) and, like achromatopsia, 'vanished' (Damasio, 1985) from the literature. Holmes wrote 'Riddoch's statement that 'the recovery of the appreciation of movements begins in the periphery of the field and extends towards central vision' is certainly incorrect', since 'In all my cases . . . the blindness was total . . . neither the presence nor the movement of any object of reasonable size could be recognized'. This would seem to leave out of account Holmes' (1918) own Case 11 in the same paper. This patient had been studied 3 months after injury, when it was found that 'there was a considerable return of vision in the periphery . . . but he was generally conscious only of the movement of the white test object, and saw it only 'as through a mist' and as a 'dirty grey colour'. Holmes conceded that there was an element of truth in Riddoch's work since 'I can . . . confirm his statements that the presence of a moving object may be recognized in which it is not perceived when stationary and in which its shape cannot be appreciated'. But there was an explanation for this, since 'I have always found that the acuity of vision in these (scotomatous) areas is considerably diminished . . . Further, colour vision is invariably affected in these areas'. Just as he thought that there was no dissociation of colour vision in striate cortex, so he explained somewhat impatiently ' . . . that the condition described by Riddoch should not be spoken of as a dissociation of the elements of visual sensation' since '. . . occipital lesions do not produce true dissociations of function with intact retinal sensibility'. With this, he despatched both Riddoch and the meagre evidence for a dissociation of function in the visual cortex.

Riddoch had tried to ascertain the position of the injuries in the brain as far as possible. He had relied mainly on x-ray examination and on notes taken during the operation. This was the best that could be done at the time. But these did not reveal the total area of the brain involved. Riddoch wrote 'At best only approximations can be made. Apart from the attempt to estimate how much brain substance has been destroyed . . . the presence or absence of sepsis has to be taken into account. Moreover, vascular lesions are never absent'. With these cautionary remarks he proceeded nevertheless to account for his findings in terms of the striate cortex alone, except where there was no dissociation of object and movement vision, i.e., where the extent of the scotoma for the two was the same. This led him to suggest ' . . . tentatively, that coincidence of the two fields and the absence of any signs of recovery are due to the injury being more extensively subcortical', even though there is some evidence to suggest that some of the wounds may have included the prestriate visual cortex. At that time, this was no cause for concern, since under the spell of Holmes and of Henschen, the striate cortex alone was regarded as the visual perceptive centre (*see* Zeki, 1990, for review). Today, the residual vision consisting of motion perception could be accounted for by subcortical mechanisms although this is not certain (*see below*).

Riddoch was thus cautious in his interpretation, explaining the relative sparing of motion vision in terms of the striate cortex alone. This was not greatly dissimilar to the way in which Marie and Chattelin (1914 – 1915) and Monbrun (1939), among others, had tried to account for achromatopsia, though they had tried to make out that the absence of colour vision could be accounted for by its greater sensitivity to, and slower recovery from, cortical onslaught. Here the history of akinetopsia differs from that of achromatopsia. Although Verrey (1888) had tried to account for achromatopsia in terms of lesions in the fusiform and lingual gyri, he had nevertheless considered these two gyri to be part of the primary visual receptive cortex. When it became clear, through the work of Henschen and others, that the lingual and fusiform gyri were outside the primary visual (striate) cortex, the resistance to the notion of a colour centre outside the striate cortex began to mount (*see* Zeki, 1990). But, except for implicating subcortical centres, nowhere did Riddoch implicate cortical areas outside the striate area. Perhaps because of this conservative explanation, perhaps because he had positive evidence (presence of movement perception in an otherwise scotomatous field rather than its total absence in an otherwise nonscotomatous field, as in achromatopsia) and perhaps because he had studied many different subjects rather than single cases, neither his evidence nor his interpretation were challenged after the initial, and apparently successful, dismissal by Holmes (1918). Instead they suffered an even worse fate. They were completely forgotten. It would be difficult to find a reference to this work in most standard books of neuro-ophthalmology published before 1983, even those which dismiss the evidence for cerebral achromatopsia. It was only many years after Riddoch's paper was published that the notion of movement dissociation was to be dismissed again, although without any reference to Riddoch's work (*see below*).

Two years after Riddoch's paper was published, Best (1919) speculated that the perception of movement and of space may be the function of areas outside the striate cortex. But he had no evidence, either clinical or experimental. Moreover, his ideas on the organization of the visual cortex were themselves a little bizarre. He believed that the sole function of the striate cortex was the fusion of the images from the two eyes. This theory was not very different from that of Ewens (1893), which Henschen (1894) was to dismiss as 'a theory in the air'. So the subject remained until Poppelreuter (1917, 1923) speculated that there must be a separation of functions in the striate cortex (calcarina). He considered that the 'sensation of movement, or change' is separately localizable, and localized, within the striate cortex. But he did not specify whether the separate submodalities were segregated according to layer, as Wilbrand (1884) and Halpern and Hoff (1929) had speculated for form, colour and the light sense (though neither had considered movement to be a submodality of vision) or whether they were localized in subareas within the primary visual cortex, as Verrey (1888) had mistakenly believed. Lacking the evidence, Poppelreuter's speculations were soon to be relegated to oblivion as well. A view of the striate cortex as the unique visual perceptive area seems to have emerged as a consensus, with Monbrun (1939) writing that '*À l'heure actuelle, tous les auteurs sont ralliés à la théorie du centre* [*visuel*] *cortical unique*'. When Holmes (1945), in his Ferrier Lecture, spoke of the striate cortex as 'a merely perceptive centre', adding that 'The perception of colour also depends on (it) . . . there is no evidence that this is subserved by any other part of the brain', he saw no reason to make a special point of emphasizing the same for motion. Riddoch's work had been truly forgotten and the speculations of Best and Poppelreuter had not been taken seriously by anyone. Only the evidence for a dissociation of colour and for a colour centre outside the striate cortex needed to be dismissed, so Holmes believed in 1945.

Just as Teuber *et al.* (1960) had believed that there is no dissociation of colour perception following cerebral lesions, so Teuber (1960) was sceptical of the dissociation of motion. In 1960, he wrote, 'It is commonly thought that cerebral lesions implicating central visual pathways tend to produce greater impairment for pattern than for motion perception. The evidence for such a statement, however, is unconvincing. It is not unexpected to find areas in defective visual fields where targets are perceived when they are in motion but not when they are stationary. The movement takes the target over a wider angular extent in the field and thus produces more stimulation. Moreover, the movement prevents the abnormally rapid fading in some (though not all) defective visual fields. Actual measurements . . . demonstrate that motion perception is impaired *pari passu* with defects in the forming of contours'. His views were unchanged over a decade later, following a detailed examination of patients with longstanding visual field defects following missile wounds to the brain, most of them sustained during the Korean War. Using both kinetic and static perimetry, Koerner and Teuber (1973) explained that a ' . . . rather surprising feature of our present findings was the thorough going association of symptoms that we encountered: once the sensitive technique of static perimetry was taken as a baseline, regional losses as defined by kinetic perimetry (and other techniques) turned out to be essentially redundant sources of information on the status of these visual fields', leading them to the conclusion that 'There was no evidence that one could dissociate detection of moving and stationary targets.' This statement was not greatly different from the earlier statement of Teuber *et al.* (1960) that 'There is thus no evidence for a genuine dissociation . . . of color and form vision'. Riddoch would not have had the satisfaction of seeing his work thus dismissed. Neither his name nor his work are referred to in this paper.

THE SPECIALIZED MOTION AREA (AREA V5) OF THE VISUAL CORTEX

At about the time that Koerner and Teuber (1973) dismissed the notion of a dissociation of motion vision from other kinds of vision, a report of a visual area lying outside the striate cortex of the macaque monkey, and apparently specialized for visual motion, was published (Zeki, 1974), a preliminary account having been published earlier (Dubner and Zeki, 1971). The area in question, area V5, lies in the posterior bank of the superior temporal sulcus and receives a direct and highly convergent input from the striate cortex, V1 (Cragg, 1969; Zeki, 1969, 1971). All cells in it are motion selective, and the overwhelming majority are directionally selective; most are not orientation selective and none is wavelength selective (Zeki, 1974). So distinctive is the area in its motion selectivity, that it was first referred to as the motion area, and subsequently called V5. It was the first visual area to provide strong evidence suggesting that the visual cortex of the primate brain is functionally specialized, with different areas processing different attributes of the visual scene, findings which were to lead to a theory of functional specialization in the visual cortex (Zeki, 1978). Within the context of this theory, it became possible to entertain the notion that lesions in specific visual areas outside the striate cortex may lead to specific visual defects, rather than a global scotoma. Since its discovery, area V5 has been the subject of numerous physiological studies, all of which are in agreement about its specialization for motion (e.g., Gattass and Gross, 1981; Albright, 1984; Movshon *et al.*, 1985). An apparently similar area in the New World owl monkey was also identified in mapping studies by Allman and Kaas in 1971. Its physiological properties were later characterized and most cells in it were also found to be directionally selective (Zeki, 1980*a*; Baker *et al.*, 1981). The homologous area in the owl monkey was called MT, and the term is commonly also used as an alternative to V5 in the Old World monkey, although I much prefer to use the term V5 when referring to the macaque monkey and to man (*see below*) and the term MT when referring to the owl monkey.

It is obvious then that by the time that Zihl *et al.* (1983) had published their single case study of akinetopsia, a considerable amount of experimental evidence had accumulated to show that there is a specialization for motion perception in the visual cortex and that the specialized area lay well outside the striate cortex. This is very different from the history of achromatopsia. Several patients with acquired achromatopsia resulting from lesions outside the striate cortex had been described before the demonstration of functional specialization in the prestriate visual cortex. There were conceptual difficulties in accepting that evidence, and hence it was dismissed (*see* Zeki, 1990, for a review). With akinetopsia, the one remarkable case came after the demonstration of functional specialization in the prestriate cortex. By 1983 all conceptual difficulties relating to the representation of a particular visual submodality in a specialized area, lying outside the striate cortex, had been removed. There seemed little reason to doubt that an akinetopsic syndrome could result from a specific lesion in a specific, motion selective, visual area. Had there been several descriptions of akinetopsia before the evidence for a functional specialization in the macaque monkey prestriate cortex had accumulated, it seems likely that the causative factor for the syndrome would have been subject to the same acrimonious debate as with achromatopsia (*see* Zeki, 1990).

THE POSITION OF AREA V5 IN MAN

The lesion in the akinetopsic patient of Zihl *et al.* (1983) was bilateral but more extensive on the left side. It included the posterior portion of the middle temporal gyrus, the retrorolandic area and the temporoparietal and occipital white matter. It also involved the subcortical white matter and the lateral occipital gyri. But it did not involve the striate or calcarine cortex. As defined recently by the technique of positron emission tomography (Cunningham *et al.*, 1990; Zeki *et al.*, 1991), area V5 occupies the temporo-parieto-occipital pit, at the boundary of areas 19 and 37, a cortical region compromised in the patient of Zihl *et al.* Nevertheless, the lesion in this patient was quite extensive and it was likely to have involved cortical tissue other than V5, in addition to white matter. Given the specificity of the visual disturbance, it seems likely that the damaged cortex outside V5 is also motion-related visual cortex. In the macaque monkey, area V5 is surrounded by satellite areas (Zeki, 1980*b*; Maunsell and Van Essen, 1983; Desimone and Ungerleider, 1986; Tanaka *et al.*, 1986; Komatsu and Wurtz, 1988). These are all involved with processing of motion-related information but in ways which differ from the role of V5. They have not yet been delineated in man as separate areas but are likely to be in the future, with the refinement of noninvasive techniques for localization. This is especially so since distinct defects related to tracking of visual stimuli have been identified in patients with lesions in cortex, in one of whom the defect was associated with akinetopsia (Thurston *et al.*, 1988). But the lesions in this latter study were relatively large. This makes it difficult to relate specific cortical loci to specific visual motion-related functions at the present time.

THE AKINETOPSIC SYNDROME IN MONKEYS

Recent evidence has shown that specific defects in motion perception can result from specific lesions in area V5 of the macaque monkey (Newsome *et al.*, 1985; Newsome and Paré, 1988). In particular, after ibotenic acid injections into area V5, monkeys are impaired in their ability to detect motion, there being a large elevation in motion detection thresholds while contrast detection thresholds are unaffected. The syndrome is short lived, however, the monkeys recovering their normal detection thresholds after a few days. The reason for this is not known but presents a contrast to the more pronounced and long-lasting akinetopsia described by Zihl *et al.* (1983). It is certain that the lesions in these controlled experiments were small compared with the relatively large lesions in the patient of Zihl *et al.* and that of Thurston *et al.* (1988). It is therefore possible that should a lesion in a monkey involve both V5 and its satellite areas, a more pronounced experimental akinetopsia would result.

THE MOTION PATHWAYS OF THE CEREBRAL CORTEX

On the face of it, the sparing of motion perception in Riddoch's 5 patients is surprising, given the fact that all had suffered from gunshot wounds, in 2 of whom the point of entry and exit of the projectile could be determined and in all of whom considerable damage to the occipital cortex and the optic radiations can be inferred from the presence and extent of the scotomas. In macaque monkey striate cortex, the directionally selective cells responsible for motion detection are situated in layer 4B and, to a lesser extent, in upper layer 6. They receive input predominantly from the magnocellular layers of the lateral geniculate nucleus, through layer 4C. The directionally selective cells in both layers project to area V5 (Lund *et al.*, 1975; Shipp and Zeki, 1989*a*). In both layers, cells projecting to V5 are separated from each other by cells projecting elsewhere, thus suggesting a degree of functional segregation for motion-selective cells (*see* fig. 1). Layer 4B also projects to V3 and to a subcompartment of area V2, the thick stripes (Shipp and Zeki, 1985; Livingstone and Hubel, 1987). In V2, directionally selective cells are concentrated in the thick stripes. (DeYoe and Van Essen, 1985; Shipp and Zeki, 1985). The thick stripes of V2 project in turn to both V3 and V5 (DeYoe and Van Essen, 1985; Shipp and Zeki, 1985, 1989*a*). These projections are quite different from those of layers 2 and 3 of V1, or of the thin stripes and interstripes of V2 (for review, *see* Zeki and Shipp, 1988).

It is conçeivable that with superficial wounds affecting layers 2 and 3 of V1 and sparing layer 4 as well as the other layers, both colour and form vision would be compromised, while motion vision would be selectively spared. In 3 of Riddoch's 5 patients with selective sparing of motion, no exit point for the projectile could be found, and it is possible that the wounds were relatively superficial, though the extent of the scotomas in each

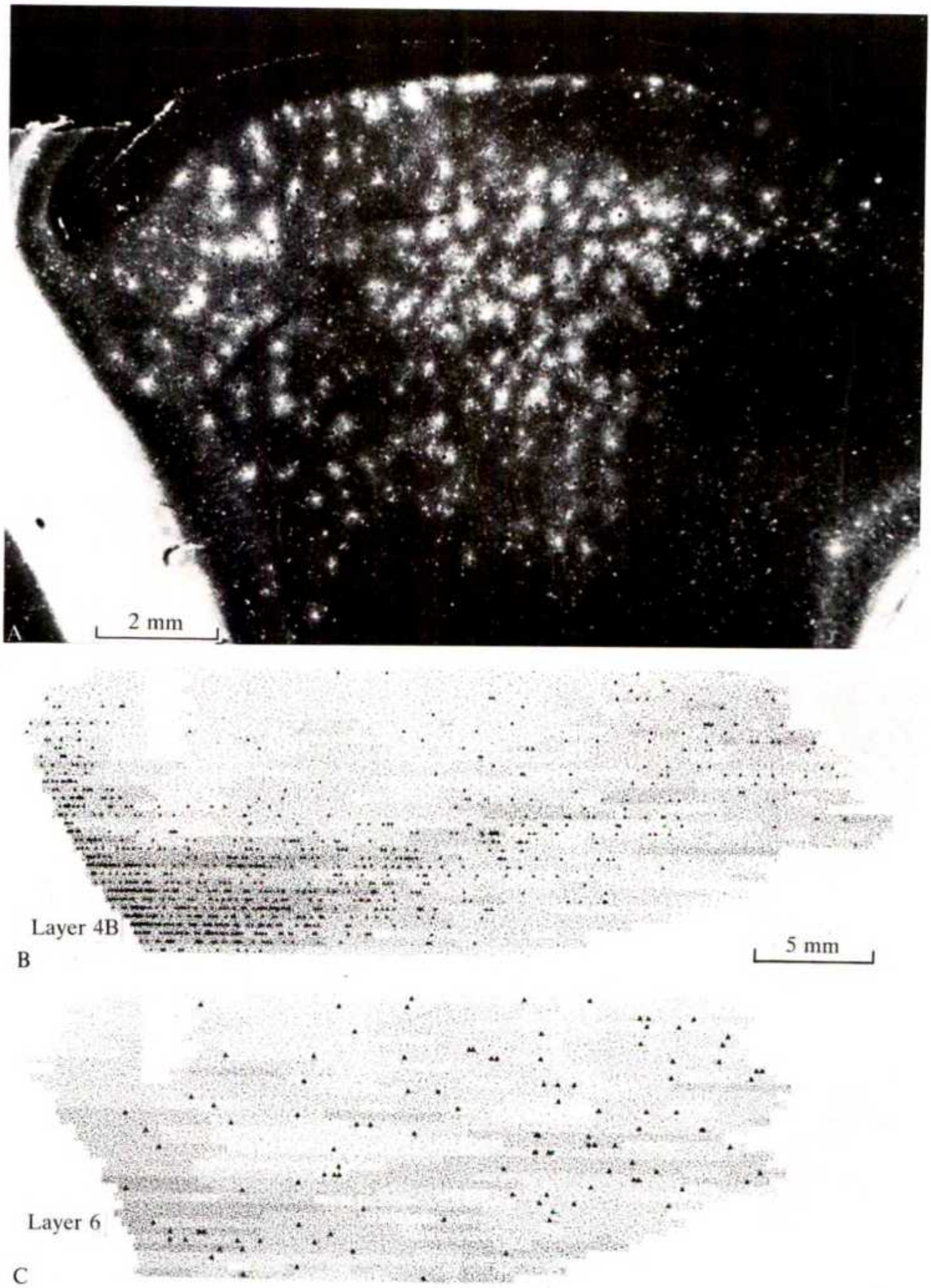

FIG. 1. A, reconstruction showing the tangential distribution of wheat-germ agglutinin horseradish peroxidase label in layer 4B of V1 following an injection of the same substance into area V5. Note the pronounced clustering of the label; B, composite computer-aided reconstruction of the distribution of labelled cells and fibres in layer 4B of V1; C, a similar reconstruction for layer 6. The reconstructions were prepared from many sections derived from the same brain. Black triangles show the distribution of cells; stippling the distribution of the return (re-entrant) fibres. Note the more widespread distribution of the return fibres. From Shipp and Zeki (1989*a*) with permission.

argues for a greater involvement. The problem is not quite so simple, however, for there is another, possibly dynamic, form system based on area V3, which also receives its projections from layer 4B (Zeki and Shipp, 1988). If layer 4B were selectively spared, at the very least some rudimentary form vision, based on the 4B to V3 system, would have been expected. Yet none of Riddoch's 5 cases had any ability to perceive forms. A more likely explanation may be that subcortical mechanisms may have been involved, although there is no evidence that this is so. In present day terms, the lesions are inadequately charted in Riddoch's patients and it is therefore difficult to account in satisfactory terms for the selective sparing of motion in these patients. There is no documented case of akinetopsia being the result of the specific involvement of the entire motion pathway from layer 4B to V5. In this, akinetopsia stands in contrast to achromatopsia where in some conditions at least, for example acute carbon monoxide poisoning or arterial insufficiency, a selective sparing or involvement of the specialized colour pathways has been suspected (*see* Zeki, 1990). It is also worth noting that the back projection from V5 to layer 4B is much more widespread than the forward, prograde, projection and encompasses not only the territory of cells in layer 4B projecting to V5 but also the territory of cells projecting to other destinations, among them V3 (figs 2, 3). Similarly, the back projections from area V5 to area V2 are also more widespread than the forward projections and, though densest in the region of the thick stripes from which V5 receives its V2 input, also include the territory of the thin stripes and the interstripes, subdivisions of V2 which do not project to V5 (Zeki and Shipp, 1988; Shipp and Zeki, 1989*a*, *b*). Given these prominent back projections, some secondary disturbances, beyond akinetopsia, might be expected after V5 lesions, but these are likely to be more subtle and are likely to have escaped notice.

It is therefore possible that the dissociation of motion vision observed by Riddoch cannot be accounted for in terms of the sparing of the relevant mechanisms within striate cortex; it may have been the consequence of intact subcortical mechanisms, or possibly the manifestation of a phenomenon analagous to residual vision or blindsight (Pöppel *et al.*, 1973; Perenin and Jeannerod, 1978; Weiskrantz, 1986; Blythe *et al.*, 1987). Present indications are that there is a small projection from the lateral geniculate nucleus to the prestriate cortex (Fries, 1981; Yukie and Iwai, 1981). Stoerig and Cowey (1989) have shown that blindsight patients can undertake wavelength descriminations, presumably through the direct pathway to V4 from the lateral geniculate nucleus. It is possible that a similar condition accounts for the phenomenon described by Riddoch. Holmes and Teuber may thus have been quite right to emphasize that the dissociation of motion vision is not the consequence of a sparing of the relevant mechanisms in the striate cortex itself. It is the insistence of both authors that visual submodalities are not dissociated in the cortex and that the striate cortex alone is responsible for visual perception that renders their conclusions untenable.

AKINETOPSIA AS A FAILURE OF A CENTRAL SYNTHETIC MECHANISM IN THE VISUAL CORTEX

Akinetopsia shares a common feature with both achromatopsia and prosopagnosia in being a condition in which the visual input to the specialized areas is intact, or largely so, while the relevant central area, being deranged, is unable to use the information reaching the cortex. In achromatopsic and prosopagnosic patients, as well as in the

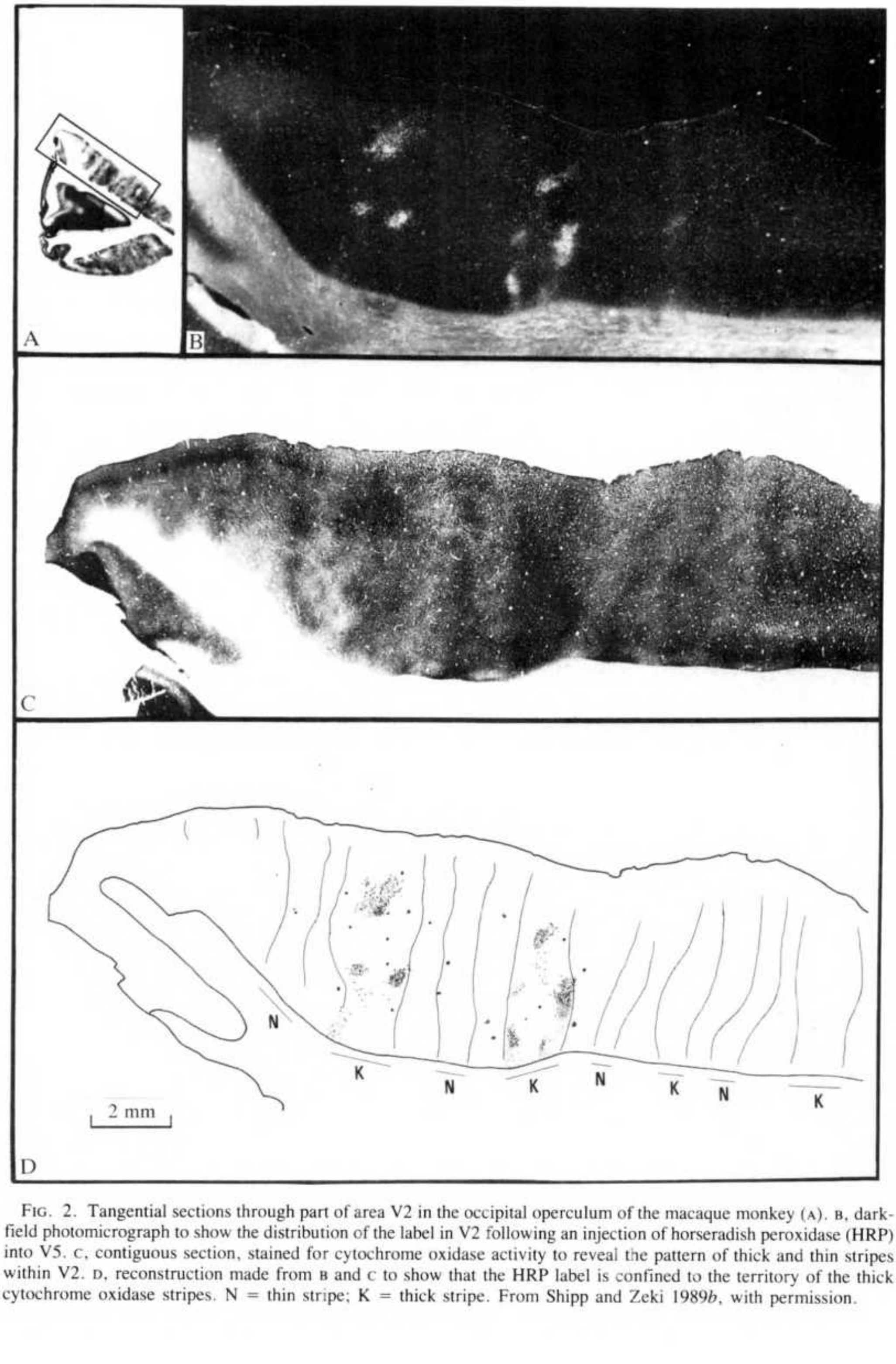

FIG. 2. Tangential sections through part of area V2 in the occipital operculum of the macaque monkey (A). B, dark-field photomicrograph to show the distribution of the label in V2 following an injection of horseradish peroxidase (HRP) into V5. C, contiguous section, stained for cytochrome oxidase activity to reveal the pattern of thick and thin stripes within V2. D, reconstruction made from B and C to show that the HRP label is confined to the territory of the thick cytochrome oxidase stripes. N = thin stripe; K = thick stripe. From Shipp and Zeki 1989*b*, with permission.

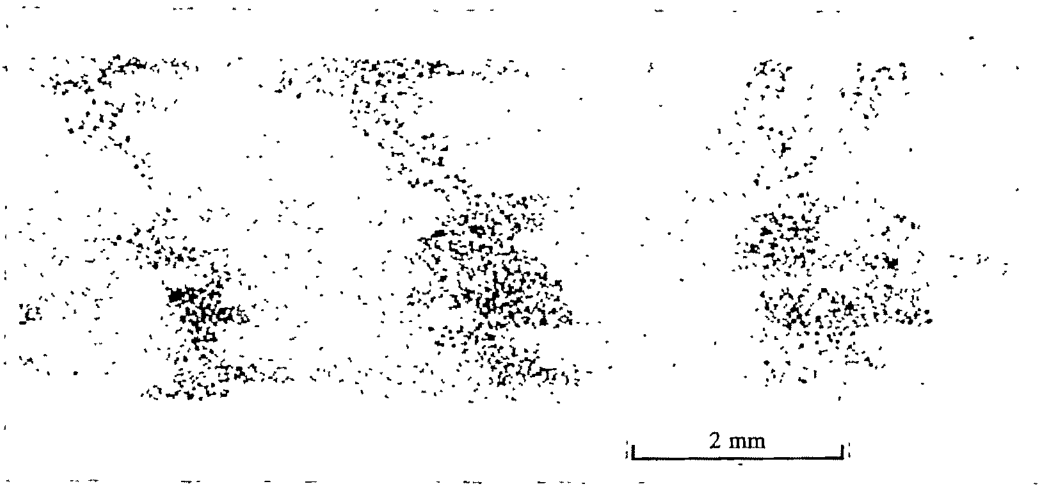

FIG. 3 Computer-aided reconstruction of the distribution of labelled cells and return fibres in tangential sections through V2, following an injection of label into V5. Conventions as in fig. 1. From Shipp and Zeki 1989*b*, with permission.

akinetopsia patient, retinal mechanisms have been found to be intact and in the latter patient even the visual evoked potentials recorded from the occipital scalp were within the normal range (Mollon *et al.*, 1980; Zihl *et al.*, 1983). Detailed psychophysical investigation of Zihl's patient has suggested strongly that all visual mechanisms up to the striate cortex are intact (Hess *et al.*, 1989), pointing to a more central failure. In all these conditions, the evidence for a failure in the central synthetic mechanism is both experimental and clinical.

In colour vision, experimental evidence suggests that the specialized cells of area V1 do not code for the colour of the stimulus, but for the wavelength (Zeki, 1983). Thus a long-wave selective cell in V1, for example, will respond to a surface of any colour provided it is reflecting a sufficient amount of long-wave light, in contrast to some cells in V4 whose responses correlate with the human perception of colours and are, within a wide range, independent of the precise wavelength-energy composition of the light reflected from the surfaces in their receptive fields. In other words, the cells of V1 are responding to component wavelengths while those of V4 are responding to the colour. It is for this reason, among others, that I have conceived of colour as being constructed by the cortex rather than being merely analysed by it (Zeki, 1984). The ingenious work of Movshon *et al.* (1985) has shown similarly that the directionally selective cells of layer 4B of V1 respond to the component motions and are unable to detect the global direction of motion of an object, which is the function of V5. In a similar way, Pallis (1955) and others have shown that a prosopagnosic patient can see all or many details of a face, such as the nose, eyes and ears, but cannot combine the features to construct a face.

CONCLUSIONS

In summary, the literature on achromatopsia contrasts with that of akinetopsia. The description of akinetopsia came at a time when the neurological world was ready to accept it, precisely because the experimental evidence made it reasonable. Had the syndrome been described before the experimental evidence for a functional specialization in the prestriate visual cortex, it would have probably been accepted, accounted for in terms of the striate cortex and then forgotten about, just as with achromatopsia. Its reverse, the presence of motion perception in blind fields, was challenged at the very beginning and then fell into oblivion. Similarly, the presence of colour vision in patients who are severely incapacitated visually was never challenged and was also relegated to oblivion. Had the causative factor for akinetopsia been attributed to a lesion outside the striate cortex, it would probably have been dismissed, just like achromatopsia and, like it, would have 'vanished' (Damasio, 1985) from the clinical literature. The remarkable contrast in the fate of these two syndromes, akinetopsia and achromatopsia, stimulates much speculation concerning the nature of the evidence that makes neurological syndromes, both those which have been described and those which await description, acceptable.

ACKNOWLEDGEMENTS

The author's work referred to above was generously supported by grants from the Wellcome Trust. I am grateful to Professor Zihl of the Max Planck Institut für Psychiatrie, Munich, for drawing my attention to some of the German manuscripts.

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(*Received March 30, 1990. Accepted May 17, 1990*)

Brain (1991), **114**, 825–841

THE NEUROLOGY OF ENDEMIC CRETINISM

A STUDY OF TWO ENDEMIAS

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SUMMARY

Endemic cretinism is the most severe manifestation of dietary iodine deficiency. Two forms of the syndrome are traditionally described: neurological and myxoedematous. Although this classification highlights the important neurological sequelae of the disorder it implies that myxoedematous cretins have an alternative mechanism. Further, the nature of the neurological deficit associated with both types of endemic cretinism has received scant attention in recent times considering that it remains a common disorder in many parts of the world.

The nature and extent of the neurological deficit found in endemic cretinism was investigated in 104 cretins from a predominantly myxoedematous endemia in western China and in 35 cretins from central Java, Indonesia, a predominantly neurological endemia. We found a similar pattern of neurological involvement in nearly all cretins from both endemias, regardless of type (myxoedematous or neurological), and of current thyroid function. Hallmarks of the neurological features included mental retardation, pyramidal signs in a proximal distribution and extrapyramidal signs. Many patients exhibited a characteristic gait. This probably reflected pyramidal and extrapyramidal dysfunction, although joint laxity and deformity were important contributing factors. Other frequently encountered clinical features were squint, deafness, and primitive reflexes. Cerebral computerized tomography (CT) revealed basal ganglia calcification in 15 of 50 subjects. The presence of basal ganglia calcification was confined to cretins with severe hypothyroidism. Otherwise, cerebral CT scanning demonstrated only minor abnormalities which did not contribute to the localization of the clinical deficits.

We conclude that the same neurological disorder is present in both types of endemic cretinism reflecting a diffuse insult to the developing fetal nervous system. These clinical findings support the concept of maternal and fetal hypothyroxinaemia, arising from severe iodine deficiency, as the primary pathophysiological event in endemic cretinism. Differences between the two types of cretinism may be explained by continuing postnatal thyroid hormone deficiency in the myxoedematous type, which results in impaired growth, skeletal retardation and sexual immaturity.

INTRODUCTION

Endemic cretinism is a syndrome of mental and physical retardation found in areas of severe iodine deficiency. Endemic cretinism has been depicted in paintings and statues since earliest times, and its association with endemic goitre was established in the late Middle Ages (Merke, 1984). Almost 40 years elapsed after the discovery of iodine (Courtois, 1813) before it was postulated that a deficiency of this element was responsible

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for endemic goitre (Chaitin, 1850). However, it was not until the clinical observations of Gull (1874), and the realization of the consequences of total thyroid extirpation (Reverdin, 1883), that a link between thyroid hormone deficiency and endemic cretinism was established.

Iodine deficiency, the most common deficiency disorder after starvation, continues to impose a substantial health burden on vast numbers of people in many parts of the world. It has been estimated that 800 million people, mainly from underdeveloped countries, suffer from iodine deficiency (Hetzel, 1987). While endemic goitre is the most common and visible marker of insufficiency of iodine, other consequences include cretinism, increased stillbirths, and increased perinatal and infant mortality (Hetzel, 1983). Endemic cretinism is the extreme manifestation of this spectrum. Although epidemiological studies have shown that endemic cretinism may be eliminated when iodine supplements are administered prior to conception or early in pregnancy (Pharoah and Connolly, 1987), many of the pathophysiological aspects of this syndrome still remain unexplained.

Most studies of endemic cretinism have concentrated on the endocrinological aspects of the disorder (*see* Delange *et al.*, 1972*a*, 1981). The neurological features of endemic cretinism have been the subject of some confusion and disagreement which undoubtedly stems from the repeated observation that two physiognomic forms of the syndrome exist. It was McCarrison (1908), during his survey of the Himalayan valleys of Chitral and Gilgit, who first divided the syndrome into two broad clinical categories, 'myxoedematous' and 'nervous' (neurological). His description forms the basis of the current classification.

Neurological cretinism is said to be characterized by euthyroidism, the presence of goitre, speech and hearing defects, together with abnormalities of gait and posture (Choufoer *et al.*, 1965). By contrast, myxoedematous cretinism is distinguished by thyroid atrophy (Thilly *et al.*, 1972), and consequently prominent features of hypothyroidism, with growth retardation and sexual immaturity. Until recently, neurological features were not reported in myxoedematous cretins, though it was recognized that they were mentally retarded.

The recent reporting of neurological signs in myxoedematous cretins has posed nosological difficulties leading to the introduction of terms such as 'intermediate' or 'mixed' cretinism (Stanbury, 1984). In a myxoedematous endemia in Zaire, DeLong (1987) found neurological deficits in 26 of 80 'typical' myxoedematous cretins. In preliminary reports from an endemia in western China where both neurological and myxoedematous cretinism exist, we have previously suggested that all cretins displayed a similar pattern of neurological signs, independent of conventional classification or current thyroid function (Boyages *et al.*, 1988; Halpern *et al.*, 1989).

If prenatal hypothyroidism is the cause of the neurological features of endemic cretinism, as suggested by Stanbury (1984), then the consequences of this intrauterine insult should be reflected equally in all types of endemic cretinism. In the light of the controversy engendered by discrepancies in the reporting of neurological signs in endemic cretinism, and recent data from animal studies showing the importance of maternal and fetal thyroid function for brain development, we studied the frequency and clinical pattern of neurological findings in endemic cretinism. In order to exclude cultural, nutritional, racial and other biases, we evaluated two different areas, a predominantly 'myxoedematous' endemia in western China (Qinghai), and a 'neurological' endemia in central Java, Indonesia (Bandung).

QINGHAI CASES, CHINA

Background

Qinghai Province lies on the Qinghai-Tibetan plateau in the central west of the People's Republic of China. In this sparsely populated, low rainfall region inhabitants mainly rely on subsistence agriculture for their livelihood. For some time, goitre and cretinism have been identified as major problems by the Ministry of Public Health in the Huong He (Yellow) River basin in the eastern region of the province, where half of the province's 4 million people are to be found (*see* fig. 1).

Epidemiological information detailing urinary iodine levels in different parts of the province is limited, as are accurate data about goitre and cretin prevalence rates. A survey of Shuobei township, Datong county, estimated that the goitre rate was between 40 and 80% and the cretinism rate as high as 10% in some villages (Qinghai Public Health Unit Report, 1978).

Subjects

Data were obtained from a sample of 104 subjects selected by local health authorities experienced in the assessment of iodine deficiency disorders. The subjects were considered to be representative of cretins in Qinghai Province. They came from 33 villages in 6 different counties within a radius of 60 km of the Institute of Endemic Diseases, and were hospitalized at the Institute near Xining, the provincial capital, for the duration of the study. All subjects lived in similar rural conditions and all were well nourished. The majority was born prior to the introduction of iodized salt prophylaxis in 1978. There were 49 males and 55 females.

For the purposes of analysis, patients were divided into euthyroid (serum thyrotropin (TSH) $\leq$ 10 mIU/l) or hypothyroid (TSH $>$ 10 mIU/l) groups. Serum was available for analysis in 102 of the 104 Qinghai cases. Fifty-nine of the patients were euthyroid and 43 were hypothyroid (Table 1).

Data collected during surveys conducted in November 1986, May 1987, December 1987 and October 1988 form the basis of the report of this endemia.

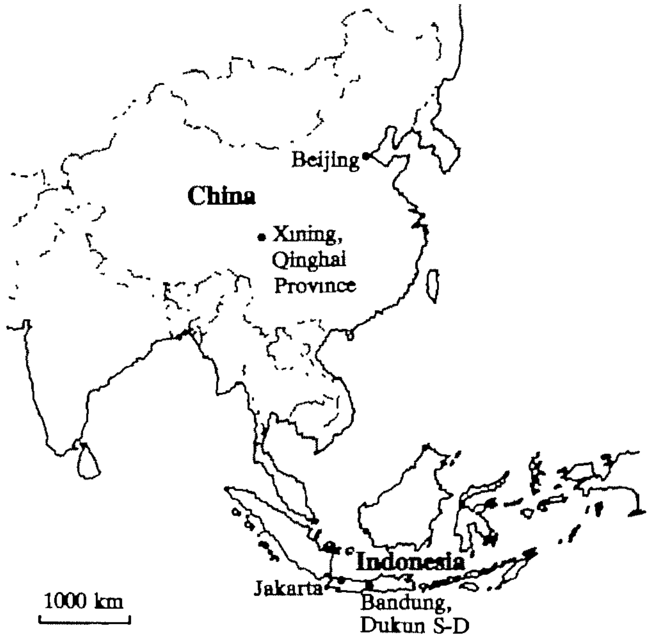

FIG 1 Map of East Asia showing the study locations in the People's Republic of China and in the Republic of Indonesia.

TABLE 1 CHARACTERISTICS OF CRETINS ACCORDING TO THYROID STATUS

	Qinghai cretins			
	Euthyroid cretins	*n*	*Hypothyroid cretins*	*n*
Age (yrs)	21.0 (18.4−23.6)	59	30.8 (27.7−33.9)*	43
Height (cm)	135.7 (131.7−140.2)	58	134.4 (128.9−135.8)	43
TSH (normal range 0.1−10.0 mIU/l)	3.8 (3.2−4.3)	59	110.6 (84.3−137.0)*	43
Free thyroxine (normal range 8.4−24.3 pmol/l)	20.7 (19.5−21.9)	58	6.8 (5.4−8.1)*	42
Goitre	16% (Grade 1, n = 8; Grade 2, n = 1)	56	7% (Grade 1, n = 2; Grade 2, n = 1)	41
Thyroid volume (ml)	12.9 (8.8−17.0)	43	3.5 (1.9−5.0)**	

	Bandung cretins			
	Euthyroid cretins	*n*	*Hypothyroid cretins*	*n*
Age (yrs)	28.4 (23.8−33.0)	27	33.3 (11.9−54.8)	3
Height (cm)	141.6 (134.3−148.9)	25	143.8 (93.5−194.1)	3
TSH (normal range 0.1−10.0 mIU/l)	4.0 (2.8−5.1)	27	40.1 (28.0−108.1)	3
Free thyroxine (normal range 8.4−24.3 pmol/l)	14.9 (13.1−16.6)	27	11.2 (1.4−20.9)	3
Goitre	15% (Grade 1, n = 3; Grade 2, n = 1)	27	33% (Grade 1, n = 1)	3
Thyroid volume (ml)	NA		NA	

* Unpaired t test, $P = 0.0001$. ** Unpaired t test, $P = 0.0005$. Results are expressed as mean (95% confidence interval). NA = not assessed.

BANDUNG HAMLET CASES, INDONESIA

Background

Goitre surveys have established the widespread prevalence of iodine deficiency in the rural regions of the Indonesian archipelago (Djokomoeljanto *et al.*, 1983). The Dukun Subdistrict, part of the Magelang Regency of Central Java, Indonesia, is a tropical, densely populated, rich agricultural area on the western slope of Mt Morapi, an active volcano. Bandung, part of the Paten village, is one of the 160 hamlets in the Subdistrict (*see* fig. 1).

An earlier survey in the Dukun Subdistrict of an adjacent village, Sengi (Goslings *et al.*, 1977), reported a low mean urinary iodine excretion rate and a cretin rate of 7.5%. A prophylaxis programme, developed by the Indonesian Health Authorities, has been in force since 1976.

Subjects

A detailed survey of the entire hamlet (423 people) by a local doctor experienced in assessing iodine deficiency disorders identified 45 people as cretins (10.6%); 35 of the 45 were available for examination at the time of the team survey in February 1988. Of the 35 cretins examined, 21 were male and 14 female. All subjects were well nourished.

In Bandung, serum was available in 30 of 35 cases: 27 of the patients were euthyroid and 3 were hypothyroid (Table 1).

METHODS

Clinical assessment

Each patient was examined independently by a neurologist (J.-P.H.) and endocrinologist (S.C.B. in Qinghai and G.F.M. in Bandung). Auxologic and clinical details were recorded on data sheets. Joint laxity was assessed according to Beighton *et al.* (1973).

Data on gait were derived from 88 Qinghai and 35 Bandung subjects. In Qinghai, videos of the gait were made to allow further analysis. Features of hypothyroidism, evidence of growth retardation, and an evaluation of the degree of sexual maturity were undertaken. Goitre was graded according to accepted criteria (Delange and Dunn, 1987).

Intelligence testing

The Hiskey Nebraska Test of Learning Aptitude (Hiskey, 1955), or the Performance Scale E of the Griffiths Mental Development Scales (Griffiths, 1984) were used to measure intelligence. Details of the methods and suitability of the tests have been discussed previously (Boyages *et al.*, 1989*a*; Yatkin and McLaren, 1970).

In Qinghai an IQ was derived for 96 of the 104 subjects (50 males, 46 females), mean age 25.9 yrs, 95% confidence interval (CI) 23.6–28.2 yrs (J.K.C.). In Bandung an IQ was derived for 28 of the 35 subjects (19 males, 9 females), mean age 28.5 yrs, 95% (CI) 24.4–32.6 yrs. Subjects were given the 'beads patterns test' of the Hiskey Nebraska Test of Learning Aptitude, and if they passed pattern 1 then the Hiskey Nebraska Test was continued. Otherwise the Scale E of the Griffiths Mental Development Scales (1984) was administered.

Audiology

A clinical evaluation of speech ability was made by doctors fluent in the subjects' language and/or local dialect. Audiometric assessment was carried out on 37 patients from Qinghai. The method has been reported elsewhere (Wang and Yang, 1985). A pure tone threshold average (PTA) was calculated from measurements made at 500 Hz, 1 kHz and 2 kHz. Audiometry was not performed in Bandung.

Radiology

X-rays of the hips and pelvis were obtained with the subjects in the weight-bearing position to assess the degree of joint deformity and laxity. Fifty patients (25 males, 25 females) from Qinghai underwent CT scanning of the head in the axial plane (Picker 1200 SX) using 10 mm slices. Contrast was not given.

Biochemistry and other procedures

Serum TSH was measured by commercial immunoradiometric assay kits (Biomedical Systems Ltd, Sydney, Australia). Serum free thyroxine levels were measured by radioimmunoassay (Biomedical Systems Ltd, Sydney, Australia). Further details of biochemical methods and the procedures used in the endocrine evaluation have been reported previously (Boyages *et al.*, 1988, 1989*b*). No ultrasonography was undertaken in Bandung.

Statistics

The results were analysed using Student's unpaired t test, one-way analysis of variance (ANOVA), and the χ^2 test, with Yates' continuity correction, if appropriate. The results are presented as the mean and the 95% confidence interval. A statistically significant difference was considered to be present at $P < 0.05$.

Voluntary consent was sought from the subjects and in all cases obtained from the subject's family. In Qinghai consent was also obtained from the local village committee and the Ministry of Public Health of Qinghai Province. In Bandung a meeting of the hamlet approved all the study procedures. The study was also approved by the District Officer and the District Medical Officer at Magelang. All study protocols were passed by the Human Ethics Committee of Westmead Hospital.

RESULTS

The degree of neurological involvement varied widely in the 139 subjects from China and Indonesia. Nonetheless, a similar pattern of neurological abnormality was seen in all cretins regardless of type (myxoedematous or neurological) and current thyroid function.

Pyramidal involvement

There was significant pyramidal dysfunction, in a proximal distribution (Table 2). Exaggeration of the tendon reflexes was more commonly encountered in the lower limbs than in the upper. All but 9 cretins from Qinghai had pathologically brisk knee jerks (91%). By contrast, the ankle jerks were brisk in only 7% of cases. When the reflexes were increased in the upper limbs it was usually in muscles supplied by the more proximal nerve roots. The findings in the Bandung hamlet subjects were similar. Pectoral and adductor reflexes tended to be present in those with marked signs of spasticity. Although hypertonus occurred in a significant proportion of patients from both endemias, it was not as prominent as hyperreflexia (Table 2). The incidence of an extensor plantar response was identical in Qinghai (17 of 103 (17%) and Bandung (6 of 35 (17%)).

Extrapyramidal features and primitive reflexes

Most subjects had a characteristic posture. They stood with their neck, hips and knees flexed, the trunk tilted forwards. These features, together with loss of arm swing and a shuffling gait, were reminiscent of Parkinson's disease. There were, however, important differences: the feet were flat and everted, the gait broad based and knock-kneed. The

TABLE 2 NEUROLOGICAL FEATURES OF ENDEMIC CRETINS FROM THE TWO ENDEMIAS

	Qinghai		*Bandung*	
	n (%)	*Total*	*n (%)*	*Total*
Pyramidal features				
Upper limb tone				
Increased wrist tone	11 (11%)	104	1 (3%)	35
Increased elbow tone	13 (13%)	104	1 (3%)	35
Lower limb tone				
Increased knee tone	21 (20%)	104	5 (14%)	35
Increased ankle tone	8 (8%)	104	1 (3%)	35
Upper limb reflexes				
Pectoral jerks	35 (35%)	103	16 (46%)	35
Brisk biceps jerks	42 (41%)	103	11 (31%)	35
Brisk brachioradialis jerks	65 (63%)	103	27 (77%)	35
Brisk triceps jerks	18 (17%)	103	3 (9%)	35
Lower limb reflexes				
Adductor jerks	49 (48%)	102	20 (57%)	35
Brisk knee jerks	94 (91%)	103	31 (89%)	35
Brisk ankle jerks	7 (7%)	103	0 (0%)	35
Extensor plantar responses	17 (17%)	103	6 (17%)	35
Extrapyramidal features				
Glabellar tap	85 (83%)	102	15 (43%)	35
Flexed posture	41 (49%)	84	20 (57%)	35
Dystonia	17 (16%)	104	1 (3%)	35
Cogwheeling	1 (1%)	104	0 (0%)	35
Primitive reflexes				
Pout	30 (47%)	64	NA	
Grasp response	61 (59%)	103	2 (6%)	35
Palmar mental response	13 (20%)	64	NA	35

NA = not assessed.

arms were often held in a curious posture with the shoulders abducted, and the elbows and wrists flexed (*see* fig. 2).

Dystonia was noted in 16% of Qinghai cases and in 1 case from Bandung (Table 2). This usually consisted of unilateral abnormalities of hand posture, with flexion of the wrist and hyperextension of the fingers at the metacarpophalangeal joints. Torticollis, with lateral neck flexion and sustained contraction of the neck and facial muscles, was seen in a minority of patients. Generalized dystonia was present in 3 subjects.

Primitive reflexes were a prominent finding among the Qinghai subjects (Table 2).

Motor and sensory findings

The low intelligence of the patients precluded a detailed examination of muscle power. No fasciculations were seen in the muscles. Bilateral lower limb wasting was seen in 10 subjects who had prominent pyramidal dysfunction. Bladder and bowel function was not affected. Sensory status was difficult to establish for the same reason. However, the preservation of tendon reflexes, the absence of cutaneous markers of peripheral sensory impairment and the relatively normal results of peripheral sensory electrophysiological testing (this will be the subject of a separate communication), suggested minimal peripheral sensory involvement.

Delay in the relaxation phase of the ankle jerk

Ninety-four subjects in Qinghai were assessed for ankle jerk relaxation phase delay: 51 were euthyroid (mean TSH 3.9 mIU/l (95% CI 3.3−4.6 mIU/l)) and 43 were

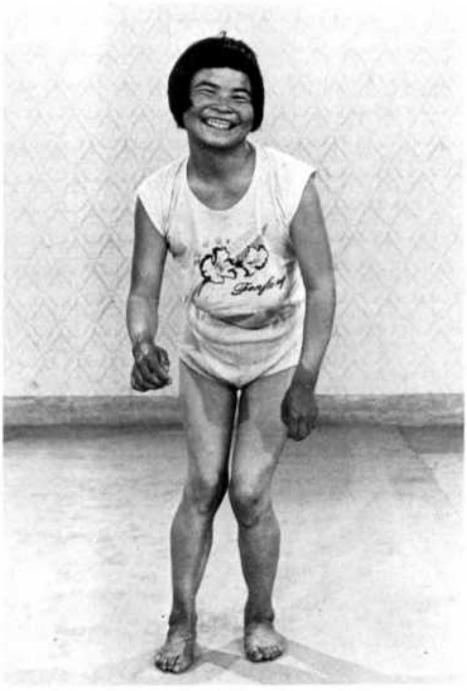

FIG. 2. Female showing characteristic postural abnormality of endemic cretinism. The hips and knees are flexed, with the trunk tilted forwards. There is knock knee, but stance is moderately wide-based. The arms are held with the shoulders abducted, and the elbows flexed. Xining, Qinghai Province, PRC, 1988.

hypothyroid (TSH 110.6 mIU/l (95% CI 84.7–137.0 mIU/l)); 11 of 51 (22%) euthyroid subjects had definite ankle jerk delay, although in the hypothyroid group only 24 of 43 (56%) had delayed ankle jerks. The specificity of ankle jerk relaxation delay was 78%, while the sensitivity was 56%. The positive predictive value of delay was 69%, while the negative predictive value was 68%. Ankle jerk delay was not seen in Bandung hamlet, where only 3 of the 35 cretins were hypothyroid. Although the prolongation of the relaxation phase of the ankle jerk was a useful predictor of the presence of hypothyroidism, it was insufficiently sensitive to be used as a diagnostic test.

Cerebellar function

Coordination, related to cerebellar function, was normal in all the subjects who could imitate the required movements. This was the case even in those cretins who were severely hypothyroid.

Psychological and psychomotor findings

All the cretins were functioning at a level of severe intellectual impairment. The mean IQ of the 96 Qinghai cretins available for testing was 29.6 (95% CI 27.06 to 32.11). In the Bandung hamlet it was 29.3 (95% CI 26.3–32.3).

Audiometry

Nearly half the patients had normal hearing (18(49%)). The mean pure tone threshold average (PTA) for the group from Qinghai was 30 dB (95% CI 24.5–35.8 dB). In those who were deaf, varying degrees of hearing impairment, ranging from a mild hearing loss to a severe hearing loss, were measured in 13 (35%), while in the remaining 6 (16%), who had a profound hearing loss, a PTA could not be calculated. Although hearing impairment, as assessed by PTA thresholds, provided only limited information about the degree of difficulty experienced by an individual listener, it nevertheless demonstrated that more than half the group had hearing dysfunction, and that its occurrence was not related to thyroid status.

Speech was normal in 9 patients (24%), and impaired in 19 (50%); 9 patients (24%) were mute.

Ocular signs

Nearly one-third (31 of 98) of the Qinghai subjects had a squint. This was nonparalytic, with one or both eyes failing to abduct fully on lateral gaze. Nystagmus was seen in only 4 subjects. The nystagmus was coarse and appeared to be related to difficulty in sustaining fixation. By contrast, only 1 subject in the Bandung sample had a squint. Pupillary size and reaction were within normal limits in both populations; 1 male hypothyroid cretin from Qinghai, with additional clinical features of a severe myopathy, had bilateral ptosis.

Musculoskeletal abnormalities

Musculoskeletal abnormalities were common and predominantly involved the weight-bearing joints (Table 3). These abnormalities showed no relationship to current thyroid function. A majority of Qinghai patients had excessive laxity of the hips, feet and ankles. In the Bandung sample, abnormalities of the feet and ankles were less prominent (Table

3). Excessive laxity of the upper limb joints was an infrequent finding. Fixed deformities of the joints and of the spine were in general confined to the Qinghai group (Table 3).

Radiographic findings

Radiological examination of the hip and pelvis demonstrated frequent abnormalities in cretins from both endemias. Abnormalities fell into two patterns: coxa valga deformity (increased flexion angle of the femoral neck); or a coxa vara deformity (decreased flexion angle of the femoral neck). In Qinghai, coxa valga was noted in 16 of 73 (22%) subjects and coxa vara in 19 of 73 (26%). In Bandung, coxa valga was noted in 7 of 31 (23%) subjects, and coxa vara in 6 of 31 (19%). A coxa valga deformity, in the Qinghai subjects, was almost always associated with the euthyroid state, and a coxa vara deformity was more commonly seen with hypothyroidism ($\chi^2 = 12.6$, df 2, $P = 0.002$). In Bandung 2 of the 3 hypothyroid subjects had a coxa vara deformity.

TABLE 3 MUSCULOSKELETAL ABNORMALITIES OF ENDEMIC CRETINS FROM THE TWO ENDEMIAS

	Qinghai		*Bandung*	
	n (%)	*Total*	*n (%)*	*Total*
Musculoskeletal feature				
Joint laxity				
Lateral knee instability	9 (9%)	104	NA	
Genu recurvatum	22 (21%)	104	5 (14%)	35
Hips				
Unilateral laxity	11 (11%)	104	1 (3%)	31
Bilateral laxity	52 (50%)	104	15 (48%)	31
Joint deformity and/or fixity				
Tibial bowing	8 (8%)	104	NA	
Scoliosis	19 (18%)	104	0 (0%)	35
Kyphosis	7 (7%)	104	1 (3%)	35
Foot abnormalities				
Fixed equinovarus deformity and subtalar laxity	17 (16%)	104	0 (0%)	35
Equinovarus ankle posture (nonfixed) and subtalar laxity	18 (17%)	103	5 (15%)	34
Subtalar laxity only	34 (33%)	103	8 (24%)	34

NA = not assessed.

These hip deformities, together with other features, including variations in the acetabular configuration, hip joint space, and epiphyseal size and shape, were consistent with an epiphyseal dysplasia. Bilateral subluxation of the hips was found in 6 of 64 (9%) cases in Qinghai and 1 of 31 (3%) cases in Bandung. Congenital dislocation of the hips was found in 1 individual from Qinghai.

Cerebral computed tomography findings

Basal ganglia calcification (BGC) was present in 15 of 50 cases on CT scanning (*see* fig. 3). Dentate calcification was an additional feature in 2 patients. The presence of BGC showed a positive relation with the hypothyroid state (one-way ANOVA, F = 5.6, $P \doteq 0.02$): those with calcification having a mean TSH of 109.5 mIU/l (95% CI

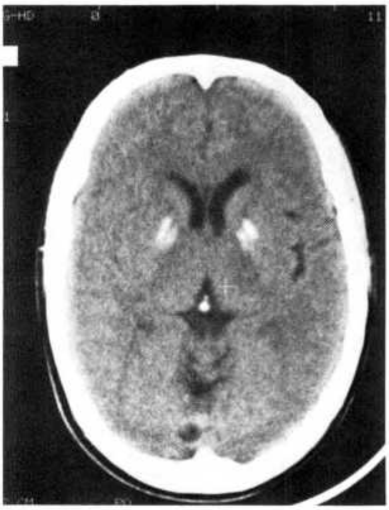

FIG. 3. CT scan of a person with endemic cretinism showing basal ganglia calcification. Mild asymmetry of the temporal lobes, manifested as a unilateral prominence of the Sylvian fissure, is also evident.

58.5 − 160.5 − 73.6 mIU/l); and those without calcification having a mean TSH of 41.6 mIU/l (95% CI 9.7 − 73.6 mIU/l). There was no significant association between the presence of BGC on CT scanning and the presence of either a dystonia or a flexed posture ((χ^2 with continuity correction = 0.02, df 1, $P > 0.05$) and (χ^2 with continuity correction = 2.25, df 1, $P > 0.05$), respectively).

Mild cerebral atrophy was found in 4 subjects. A further 4 subjects had areas of focal cerebral atrophy; 3 of the 4 scans were consistent with areas of previous infarction, presumably secondary to birth trauma. Three patients had evidence of mild cerebellar atrophy; 2 of these 3 cases were euthyroid. Overall, the CT findings were surprisingly few considering the severity of the neurological disturbance in this group of subjects. We were unable to find any correlation between the CT abnormalities and the clinical signs.

Gait disturbance

A characteristic gait disturbance was found in most subjects, with disability ranging from a mild disturbance of posture and rhythm to an inability to walk. In its mildest form, subjects walked on a widened base with a slight waddle and with reduced arm swing. These features were accentuated in more severely affected individuals. In these, the gait was slow and shuffling, turning was effected in series, all movements tended to be stiff and jerky, and there was a pronounced waddle. In many individuals the arm swing was asymmetric or absent. Some were only able to walk with assistance and 2 could not walk at all. The frequency of the main gait features is shown in Table 4.

The knock knees, wide base and pronounced waddle probably resulted from an excessive laxity and deformity of the lower limb joints, leading to a collapse of the structural platform of locomotion. Nonetheless, the final effect of joint deformity on gait was the same regardless of the hip abnormality, since a deviation from the optimal angle, irrespective of the direction of deviation, results in the loss of mechanical advantage

TABLE 4 GAIT CHARACTERISTICS OF ENDEMIC CRETINS FROM THE TWO ENDEMIAS

	Qinghai		*Bandung*	
	n (%)	*Total*	*n (%)*	*Total*
Abnormal gait	76 (86%)	88	24 (69%)	35
Lower limb				
Flat feet	77 (88%)	88	16 (46%)	35
Feet everted	62 (71%)	87	8 (23%)	35
Feet inverted	6 (7%)	84	0 (0%)	35
Wide base	46 (53%)	86	18 (53%)	34
Knock knees	47 (54%)	87	20 (57%)	35
Knees flexed	40 (48%)	84	20 (57%)	35
Hips flexed	41 (49%)	84	20 (57%)	35
Trunk				
Trunk tilt	42 (51%)	82	8 (23%)	35
Trunk hyperextension	26 (31%)	84	22 (63%)	35
Slow turning	43 (51%)	85	19 (54%)	35
Shoulder sway	23 (62%)	37	NA	
Upper limb				
Reduced arm swing	63 (75%)	84	20 (57%)	35
Abducted/flexed arm posture	23 (30%)	77	10 (29%)	35

NA = not assessed.

at the hip. Where cooperation was possible, cases with waddle invariably demonstrated a positive Trendelenberg test. This was due to excessive hip laxity and hip joint deformity leading to a loss of mechanical advantage at the hip. Furthermore, the normal smooth transfer of motion and weight of the torso were disrupted by the presence of ankle deformity and subtalar laxity. The result was a partially flexed leg, rather than a fully extended one, striking the ground at the end of the gait cycle, with the entire foot not just the heel.

DISCUSSION

The two endemias described in this study were widely dissimilar geographically and racially, one was predominantly 'myxoedematous' and the other 'neurological'. Yet the pattern of neurological abnormality in subjects with cretinism from the two endemias was the same. The clinical neurological picture comprised a proximal distribution of pyramidal signs, extrapyramidal dysfunction, a characteristic gait abnormality, intellectual impairment, deafness, primitive reflexes and squint. Our findings conflict with the traditional dualistic classification of endemic cretinism, where the 'neurological' type is viewed as a separate manifestation of the syndrome from the 'myxoedematous' type.

Based on these observations, it can be concluded that the clinical picture of endemic cretinism results from an interaction of two pathophysiological events, both resulting from iodine deficiency, but acting at different times in the development of the fetus and neonate. The first event represents a lack of thyroid hormone in utero, resulting in the neurological manifestations of the condition. The second variable, progressive thyroid atrophy, determines the duration and severity of continuing hypothyroidism after birth, which is reflected clinically as growth retardation, sexual immaturity and persisting signs of thyroid hormone deficiency. Both components play a role, and explain the frequent overlap of clinical signs.

A careful review of the literature shows that the findings of our study are not inconsistent with previous published data, and that few endemias could be reconciled with the traditional classification of endemic cretinism (*see* Lobo *et al.*, 1963; Ibbertson, 1971; Delange *et al.*, 1981; Boyages *et al.*, 1989*a*). Deafness, mental retardation, and pyramidal and extrapyramidal dysfunction have been reported from different endemias in varying frequency and severity (*see* Choufoer *et al.*, 1965; Goslings *et al.*, 1977; DeLong *et al.*, 1985). These findings were explained as representing two different ends of a continuous spectrum, from 'neurological' to 'myxoedematous' cretinism.

The myxoedematous endemia of Zaire is widely quoted as the paradigm supporting the concept of two distinct types of endemic cretinism. Nevertheless, the perception that neurological features, apart from intellectual impairment, were extremely infrequent, is untenable based on observations reported at that time. For instance, Dumont *et al.* (1963) found deaf mutism in 14%, and spastic gait and muscle spasticity in 5.7% of myxoedematous cretins from the Uele district. In another major report from Zaire, 11% (11 of 99) of cretins in Idjwi Island were clinically euthyroid, of whom 7 exhibited 'spastic diplegia' (Delange *et al.*, 1972*b*). In the remaining 88 hypothyroid cretins, a positive Babinski sign was elicited in 45% (5 of 12) subjects in whom it was sought. Recently, in the Ubangi district of Zaire, another area of myxoedematous cretinism, DeLong (1987), a paediatric neurologist, cast doubt on these initial observations by demonstrating neurological deficits in 33% (26 of 80) of 'typical' myxoedematous cretins. In addition, he found predominant neurological features similar to those found in 'neurological' cretinism in a further 23% (18 of 80) during the same study.

Several factors may be responsible for the wide divergence in the reporting of neurological abnormalities from Zaire. Undoubtedly, observer error may have been a factor in reporting the frequency of the neurological features (Delange *et al.*, 1972*b*; DeLong, 1987). Secondly, selection pressures may have led to a particularly high mortality rate in myxoedematous infants with severe neurological deficits, resulting in the neurological features of the surviving group being less obvious. Iodine deficiency is associated with an increased incidence of abortion, and perinatal and infant mortality (McMichael *et al.*, 1980). In addition, Querido (1975) reported that severely neurologically affected cretins in the Mulia Valley of western New Guinea tended to die by the age of 6 yrs, and McCarrison (1917) observed that myxoedematous cretins were particularly susceptible to infectious diseases, 'the large majority succumb in early life'. Lastly, some myxoedematous cretins may actually reflect severe postnatal (juvenile) hypothyroidism, cases of which would not be expected to have the same pattern of neurological damage. However, this concept is not supported by the results of neonatal screening from these areas which revealed markedly elevated TSH levels (Thilly *et al.*, 1978), and radiological evidence of epiphyseal dysplasia in the femoral head of myxoedematous cretins, as both of these observations point to a prenatal onset of hypothyroidism.

The pattern of neurological damage in endemic cretinism is distinctive and confined to areas of severe iodine deficiency. There are at least three possible mechanisms by which a lack of elemental iodine may adversely affect brain development. The first is that iodine, independent of thyroid hormone, may have an essential role in the growth and development of the human brain. Alternatively, iodine deficiency may cause maternal hypothyroidism and thereby result in deficient transfer of thyroid hormone across the placenta to the developing fetus and damage the fetal brain in early pregnancy. Iodine deficiency, due to an insufficient supply of iodine from the mother, may have a direct effect on the fetal thyroid and induce hypofunction of the gland. The last two hypotheses depend on experimental data showing that the human brain is sensitive to the actions of thyroid hormone in the prenatal period. Finally, these mechanisms may not act in isolation but may synergize to produce damage to the developing nervous system. The resolution of the question as to which of these mechanisms is paramount required an experimental model, and to this end, studies have been carried out in a number of animal species.

Studies in sheep and rat have shown that both maternal and fetal thyroxine is important for normal brain development in the fetus. Combined maternal and fetal hypothyroxinaemia, produced either by surgery or iodine deficiency in sheep, has led to severe neurological defects in the sheep fetus, acting through hypothyroidism and not iodine deficiency alone (Hetzel *et al.*, 1983). These studies have shown that thyroid hormone of maternal origin crosses the placenta in significant amounts (Obregon *et al.*, 1984). Furthermore, recent experimental studies (Morreale de Escobar *et al.*, 1989) have shown that placental transfer of maternal thyroxine has a protective effect on the fetal brain and other tissues when fetal thyroid function is impaired. Fetal hypothyroxinaemia has been demonstrated in human fetuses from regions of severe iodine deficiency from the fourth month of gestation (Liu *et al.*, 1989). Low maternal serum thyroxine concentrations are frequently encountered in areas of severe iodine deficiency and have been found to correlate with poor intellectual outcome in offspring (Pharoah *et al.*, 1984). In addition, recent studies of neonates with severe congenital hypothyroidism have demonstrated substantial transfer of thyroxine from the mother to the infant up until term, suggesting that the transfer is important in mitigating the effects of fetal hypothyroidism (Vulsma *et al.*, 1989).

The neurological findings in endemic cretinism impose some limits on the timing of the intrauterine insult to the developing brain. The manifestations of iodine deficiency involve auditory, extrapyramidal and intellectual function which indicate damage to the cochlea, basal ganglia and cerebral cortex. These structures form predominantly in the second trimester, well after the period of brain organogenesis, which occurs in the first trimester. The second trimester is the time of maximal brain growth, corresponding to neuronal cell multiplication and migration (Dobbing, 1970, 1974). Based on this timetable of neural development in the fetus, DeLong (1987) proposed that the primary pathogenic event occurs during the second trimester, a time when the ontogenic development of the thyroid and brain appear to be crucially linked. This hypothesis is supported by the clinical findings of our study where severe intellectual impairment was found in all cretins, and significant hearing impairment was measured in half of those examined regardless of current thyroid status.

Extrapyramidal signs consistent with basal ganglia involvement are an integral feature of the neurological deficit of endemic cretinism. A flexed posture, slow turning, reduced arm swing, lack of inhibition of blink to glabellar tap, and involuntary smiling and grimacing were common findings. DeLong (1985) and DeLong *et al.* (1987) have also drawn attention to extrapyramidal involvement in endemic cretinism, describing prominent truncal rigidity, bradykinesia and dystonic posturing of the head and neck. Along with other observers (Lobo *et al.*, 1963; Choufoer *et al.*, 1965) DeLong *et al.* (1985) noted the characteristic posture of flexion of the hips and knees with forward tilt of the trunk. This posture is reminiscent in many ways of the stance in Parkinson's disease. In Parkinson's disease it appears that rigidity is responsible for the characteristic flexed posture, although the cause of that rigidity remains controversial (*see* Burke, 1987). Unlike Parkinson's disease the reflexes in endemic cretinism are brisk.

Dystonia, another manifestation of basal ganglia dysfunction, was a clinical feature of endemic cretins from Qinghai. This varied from a focal dystonia of the distal upper limb to generalized dystonia. Although basal ganglia calcification (BGC) was found in just under one-third of the Qinghai cretins scanned, it cannot be invoked as a cause or marker of basal ganglia dysfunction, as its presence showed no relationship with the postural abnormalities and dystonia. In Qinghai, BGC was confined to cretins with severe hypothyroidism. In our study, BGC may be explained by the action of long-standing severe hypothyroidism on calcium homeostasis.

Hypothyroidism acquired in adulthood has traditionally been associated with a cerebellar disturbance. Along with previous investigators we found no evidence of this in the cretins (e.g., Lobo *et al.*, 1963; DeLong *et al.*, 1985). This may reflect the fact that the development of the cerebellum in the human mainly occurs in the postnatal period, the neurological insult in endemic cretinism being primarily prenatal (Dobbing 1970, 1974).

Nonetheless, there are significant postnatal sequelae of iodine deficiency, as hypothyroidism has profound effects on sexual maturation, growth and bone development (Thilly *et al.*, 1972; Querido *et al.*, 1974). Postnatal hypothyroidism is responsible for the striking physiognomic differences of the disorder. The gait of endemic cretinism, which has long been recognized as a distinctive integral feature of the disorder, provides an illustration of the interplay of pre- and postnatal hypothyroidism. It seems likely that the gait results from a combination of pyramidal and extrapyramidal dysfunction (prenatal effects), together with joint deformity (postnatal effect), and joint laxity. The 'cerebral diplegia' and resultant 'peculiar stiffness of gait' of McCarrison (1908, 1917) is an inadequate description of the gait.

Radiological examination suggests that the joint laxity is due to an underlying epiphyseal dysplasia (Shapiro, 1987). In our study, hip x-rays showed abnormalities of femoral alignment in just under half the cases from both endemias. A coxa valga deformity was almost always associated with the euthyroid state, while a coxa vara deformity was more commonly seen with hypothyroidism. The correlation of the type of hip deformity with thyroid status is strong evidence of the effect of postnatal thyroid hormone deficiency on bone modelling. Presumably muscle imbalance from long-standing spasticity due to ineffectively opposed spastic adductor and iliopsoas muscles is the cause of the functional coxa valga of endemic cretinism, in a manner similar to the coxa valga deformity of cerebral palsy (Sauser *et al.*, 1986). The characteristic hip abnormality of congenital hypothyroidism or acquired juvenile myxoedema, where there is delayed appearance and fusion of all epiphyses, is a shortening of the femoral neck and coxa vara (Greenfield, 1986). Whether coxa valga or coxa vara occurs in hypothyroid cretins is determined by the balance of the competing influences of spasticity (prenatal effects) and hypothyroidism (postnatal effects).

Endemic cretinism is the most dramatic and poignant manifestation of iodine deficiency.

The disorder serves as a human model for the understanding of prenatal thyroid hormone action on fetal brain development. Although the exact pathophysiology of various aspects of the syndrome of endemic cretinism is still not totally resolved, based on the present observations the traditional separation of the endocrine and neurological forms of the disease is illogical, and has served to obscure advances in the understanding of maternal and fetal thyroid hormone homeostasis. We (Boyages *et al.*, 1988) recently suggested that prenatal thyroid hormone deficiency, from maternal and fetal sources, secondary to severe iodine deficiency, results in the neurological sequelae of the disorder. This process is common to all forms of endemic cretinism. In addition, a second independent process that affects a minority of individuals, progressive thyroid atrophy, acts to determine the severity and duration of postnatal hypothyroidism. The latter process is responsible for the diverse clinical manifestations of cretinism (Boyages *et al.*, 1988, 1989*c*).

ACKNOWLEDGEMENTS

Jean-Pierre Halpern and Steven C. Boyages are recipients of National Health and Medical Research Council (NHMRC) of Australia postgraduate medical scholarships. In China the work was supported by a cooperative project between the Ministry of Public Health of the People's Republic of China and the Australian International Assistance Development Bureau. In China and in Indonesia the work was supported in part by the NHMRC of Australia.

The authors also wish to thank the following individuals for their collaboration and assistance. Ms Frances Gibson, Dr James Jupp, Ms Lidija Nemitischenko, and Professor John Anthill who assisted in the psychometric testing. Dr Faisah Yasin assisted greatly in the collection of data, and facilitated our stay in the Dukun Subdistrict, Indonesia. Dr Peter Carr reported on all the cerebral CT scans and x-rays, while Dr M. Y. Yu supervised the radiographic procedures. Audiometry was kindly performed by Professor Wang You Yu. Drs Wang Zheng-Hua, Jin Chen-En and all the staff of the Institute of Endemic Diseases, and the Qinghai Province Ministry of Public Health, Xining, PRC, supported our efforts enthusiastically. Mr Peter Heldorf produced and directed the videotape material in October 1988.

A preliminary report of these results was presented at a meeting of the Association of British Neurologists in London in September 1989, and at the XIVth World Congress of Neurology, New Delhi, India, in October 1989.

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(Received November 28, 1989 Revised April 17, 1990. Accepted May 22, 1990)

Brain (1991), **114**, 843–853

CHANGES IN SIZES OF CORTICAL AND LOWER MOTOR NEURONS IN AMYOTROPHIC LATERAL SCLEROSIS

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SUMMARY

It has been suggested that the degeneration of lower motor neurons in amyotrophic lateral sclerosis (ALS) is a transneuronal event, secondary to the loss of corticospinal and corticobulbar neurons. In an attempt to test this hypothesis, the cross-sectional areas of pyramidal cells in layer 5 of the foot and tongue areas of the precentral gyri were measured in 12 cases of the classical sporadic form of ALS, and in 10 control subjects. The areas of motor neurons in the hypoglossal nuclei and in the ventral horns of segment L4 of the spinal cord were also measured. The number of neurons per 20 μm section of ventral horn or hypoglossal nucleus provided a more reliable index of severity of lower motor neuron loss at the time of death than did a semiquantitative score derived from clinical observations.

Cortical neurons and lower motor neurons were significantly smaller in the cases of ALS than in the controls. In the cortex this change included, but was not confined to, the largest neurons. These observations indicate that shrinkage precedes neuronal death. There was no correlation, positive or negative, between the numbers of surviving lower motor neurons and the mean sizes of pyramidal cells in layer 5 of the corresponding areas of the precentral gyri. The absence of such a correlation indicates that functionally related cortical and lower motor neurons probably degenerate independently, and not from a transsynaptic effect. Neuronal shrinkage has been observed in other diseases in which interconnected systems of neurons degenerate. The possible association of shrinkage with cytoskeletal degradation is discussed.

INTRODUCTION

There are several diseases in which functionally connected systems of neurons degenerate. It has been speculated that the pathological processes spread from one neuron to another. This could occur by axonal transport and transsynaptic transfer of pathogens, or as a result of loss of essential trophic factors that are normally secreted by pre or postsynaptic cells (*see* Saper *et al.*, 1987). In the classical sporadic form of amyotrophic lateral sclerosis (ALS), motor neurons die in the ventral horns of the spinal grey matter and the motor nuclei of cranial nerves, and there is a depletion of large pyramidal neurons, known as Betz cells, in the primary motor cortex of the precentral gyrus. It is believed that the axons of Betz cells are presynaptic to motor neurons (*see* Brodal, 1981; Rothwell, 1987; Nieuwenhuys *et al.*, 1988). In view of evidence of spinal motor neuron degeneration following cerebral infarction (Goldkamp, 1967; McComas *et al.*, 1971; Botez, 1972; Brown, 1984), we have suggested that death of cortical neurons precedes transneuronal

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atrophy of lower motor neurons in ALS (Hudson and Kiernan, 1988). This hypothesis would be supported by finding degeneration in parts of the primary motor area connected with spinal segments or cranial nerve nuclei in which the lower motor neurons were not affected by the disease at the time of death. Conversely, an association of lost lower motor neurons with normal motor cortex would militate against the idea of descending transneuronal degeneration, and might indicate that death of Betz cells follows the loss of motor neurons.

The surviving spinal motor neurons in ALS have been counted (Tsukagoshi *et al.*, 1979, 1980) and examined by electron microscopy (Hirano *et al.*, 1984; Kusaka and Hirano, 1985; Gold, 1987) and with silver (Nakano and Hirano, 1987) and Golgi (Kato *et al.*, 1987) methods. The changes in the cerebral cortex have also been examined qualitatively, using the Golgi and other light microscopic methods (Hammer *et al.*, 1979; Horoupian *et al.*, 1984; Udaka *et al.*, 1986). These investigations have shown that fragmentation and loss of dendrites precede degeneration of the somata of motor neurons and Betz cells. It is still not known whether degeneration of the corticospinal axons proceeds anterogradely (Lawyer and Netsky, 1953) or retrogradely (Davison, 1941; Brownell *et al.*, 1970). Degenerating Betz cells have been seen in the absence of visible degeneration of the corticospinal tracts (Udaka *et al.*, 1986), but the number of Betz cells is sufficient to give rise to only about 1% of the corticospinal fibres in the medullary pyramid (Lassek, 1941; Nyberg-Hansen and Rinvik, 1963). The cells of origin of motor corticobulbar fibres in man are not known, though some of these axons end within motor cranial nerve nuclei (Kuypers, 1958) and are presumably equivalent to the direct corticomotoneuronal fibres present in the corticospinal tracts (*see* Brodal, 1981; Rothwell, 1987; Nieuwenhuys *et al.*, 1988).

There has been no previous quantitative study attempting to relate the changes in motor neurons to those in cortical pyramidal cells in ALS. Such an investigation is now presented.

MATERIAL AND METHODS

Postmortem material was collected from 12 cases of ALS. All had the classical sporadic form of the disease; none showed signs of dementia, parkinsonism or other diseases of the nervous system. The youngest patient died at the age of 47 and the oldest at 76 yrs (*see* Table 1) from respiratory paralysis. Brains and spinal cords were also collected from 10 individuals, aged 41–89 yrs, with no clinical evidence of abnormality of the nervous system. Clinical records and pathologists' reports were available for all these cases. The causes of death of the control subjects were myocardial infarction (6), lobar pneumonia and septicaemia (1), aortic aneurysm (1), aortic stenosis (1) and multiple myeloma (1).

The brains and spinal cords were removed 2–24 h after death and fixed by immersion in phosphate-buffered 4% formaldehyde for 2–3 wks before dissection. Segmental levels in the caudal part of the spinal cord were identified by the criteria of Hirano *et al.* (1980). Pieces 2 mm thick were taken from segment L4 for sectioning in the transverse plane. A 2 mm slice was also taken from the medulla, at the level of the caudal one-third of the olive. Two specimens were taken from the precentral gyrus of each cerebral hemisphere: 1 from the medial surface (ankle and foot area), and 1 from the lower end of the gyrus, near the lateral sulcus (tongue area). Each specimen included the whole width of the gyrus, and was orientated so that sections could be cut in a plane transverse to its long axis, and perpendicular to the pial surface. This ensured that the pyramidal cells would be cut in their long axes. The central nervous tissue was subsequently stored in 4% formaldehyde for up to 3 yrs, and further pieces were taken for histological study as needed. Numerous other specimens, from the nervous system and from other organs, were taken for routine pathological examination.

Paraffin sections 20 μm thick were stained by the iron-chromoxane cyanine R method for myelin (Kiernan, 1990, pp. 319–320) and counterstained with 0.5% neutral red, pH 4.0 to show Nissl substance and nuclei. These were used for counting and measuring cells. To identify pathological changes within neurons, sections 6 μm thick from the same blocks were stained either with erythrosin and toluidine blue (the Mann-Dominici method, after Gabe, 1976, pp. 233–235) or by Lillie's azure-eosin method (Kiernan, 1990, pp. 101–102). The slides were given code numbers in the hope that this would eliminate subjective bias, but it was not possible to conceal the conspicuous abnormalities in the spinal cords and cranial nerve nuclei of the diseased specimens.

With the aid of a camera lucida, sections through the ventral horns of the spinal grey matter and the hypoglossal nuclei in the medulla were drawn on paper at a linear magnification of ×20. Motor neurons were marked and counted. Individual motor neurons with their nucleoli in the plane of section were traced at ×300. Pyramidal neurons of layer 5 in sections of cerebral cortex were also drawn at ×300. Layer 5 was identified as the zone containing large pyramidal cells that was deep to a tangential band of myelinated fibres constituting the inner band of Baillarger, which is in layer 4. All triangular or pear-shaped cells with nucleoli were drawn. It was assumed, for standardization, that the nucleolus was in the centre of each neuron. The areas of the drawn profiles were measured, using a Micro-plan II digital image analyser (Donsanto Corp., Natick, Massachusetts). Abundance of motor neurons was recorded as the number of the cells per 20 μm section of anterior horn or hypoglossal nucleus at each level.

For each of the two groups (control and ALS), the mean, standard deviation (SD) and standard error of the mean (SEM) were determined for the number of motor neurons per 20 μm section of ventral horn or hypoglossal nucleus (the left and right sides were pooled). For each type and location of motor and pyramidal neuron, the mean area with its SD and SEM were calculated for each individual subject In order to look for changes confined to the largest cortical neurons, means and related statistics were also determined for a selected sample comprising the highest 10% of the measured neuronal areas in each cortical section. Group means of the mean areas of motor neurons and pyramidal cells were calculated for comparison of the cases of ALS with the controls. Means in the two groups were compared by Student's t test, with Bessel's correction for small samples. Within the group of cases of ALS, associations were sought between clinical severity of paralysis of the muscles moving the ankle or tongue, or of motor neuron population in L4 or the hypoglossal nucleus, with sizes of pyramidal cells in the corresponding regions of the primary motor cortex. Simple correlation coefficients and least squares regression equations were calculated, and their significances determined, following Zar (1984). For all statistical comparisons, a value of $P < 0.05$ was considered unlikely to be due to chance.

RESULTS

The rate of progression of the paralysis and the groups of muscles first affected were variable. The duration of the disease ranged from 5 to 35 months from the onset of the first perceived weakness or fasciculation. The clinical data for the 10 patients are summarized in Table 1, which shows that the facial and oropharyngeal musculature was spared or only slightly weakened in some cases. All patients had moderate to complete upper and lower limb paralysis. The first symptom affecting a limb was usually unilateral, but by the time of death there was little difference between the severity of paralysis on the left and right sides.

Qualitative observations

The only abnormality seen regularly in the CNS of the control subjects was the presence of numerous corpora amylacea in the white matter of individuals more than 60 yrs old. In one 88-yr-old brain, there was also granulovacuolar degeneration, most marked in the cells of the oculomotor nucleus. Corpora amylacea, which were also seen in the older cases of ALS, are attributed to ageing, and are not believed to be associated with any functional disability (Tomlinson and Irving, 1977; Weller, 1984). Granulovacuolar

TABLE 1. CLINICAL FEATURES OF THE AMYOTROPHIC LATERAL SCLEROSIS CASES

			*Severity of paralysis shortly before death***						
Age (yrs) at time of death, sex	*Site of first weakness noticed by patient**	*Duration initial weakness to death (mos)*	*Shoulders and upper arms*	*Forearms and hands*	*Hips and thighs*	*Lower legs and feet*	*Face*	*Pharynx*	*Tongue*
52 M	R leg	26	1	1	2	2	3	3	3
54 M	L upper arm	32	2	2	3	3	2	3	2
55 F	L lower leg	11	3	4	3	1	5	5	5
57 F	R leg and foot	28	3	3	2	1	5	5	5
57 M	Dysphagia	35	2	1	2	3	2	1	1
58 F	Generalized	18	4	3	4	2	4	4	4
60 F	R arm	33	0	0	2	3	4	4	4
61 F	Dysarthria	17	0	1	0	0	1	0	1
68 F	R leg	5	3	3	2	1	3	3	2
70 F	L leg	20	4	3	2	1	4	4	4
72 M	Generalized	7	3	3	4	4	5	5	5
78 F	R foot and leg	18	4	4	3	3	5	5	5

* 'Leg' and 'arm' refer to the whole limb. ** 5 = normal; 0 = no detectable function

degeneration was seen in the oculomotor and hypoglossal nuclei of 2 of the ALS cases. This is another change associated with age, although it is also seen in various neurodegenerative disorders (Okazaki and Scheithauer, 1988). The cytoplasmic inclusions do not resemble the Hirano and Bunina bodies seen in some motor neurons in ALS (Hirano *et al.*, 1980).

Pathological changes characteristic of ALS were seen in the cervical, thoracic and lumbosacral spinal cord in all the clinically diagnosed cases of the disease. Motor neurons were scanty in the ventral horns, although the outline of the grey matter was of normal size. The spaces between surviving neurons contained fibrillary eosinophilic material and nuclei of glial cells. Occasional neuronophagic figures and ghost cells were seen in most sections. The perikarya of the surviving motor neurons were often abnormal. Diffuse cytoplasmic basophilia replaced the normally large Nissl bodies, and some of the cells contained eosinophilic inclusions. Similar changes were seen in the trigeminal and facial motor nuclei, nuclei ambigui and hypoglossal nuclei in many of the cases of ALS, but in this site there was much more variation among individuals. No abnormalities other than occasional granulovacuolar degeneration were seen in the ocular motor nuclei. The nucleus of Onuf, seen in segments S2–S3 from 6 of the control subjects and 6 ALS cases, was conspicuous for the normal appearance of its neurons, which were smaller and more densely packed than the surrounding motor neurons.

The intensity of the myelin stain was reduced in the dorsal half of the lateral funiculus in the spinal cords of patients with ALS, but the appearances were inconsistent at different segmental levels. Variable staining intensity was also seen in the spinal white matter of most of the control subjects. Tomlinson and Irving (1977) have made the same observation, and we agree with them that 'pallor' is not a reliable sign of degeneration of the lateral corticospinal tract. Convincing signs of degeneration were never seen in the medullary pyramids or in the middle part of the basis pedunculi in sections stained to show normal myelin.

Abnormalities in the primary motor area of the cerebral cortex were less conspicuous than those in the spinal cord. The principal change with ALS was a shortage of neurons in the deeper layers of the cortex. Consequently, the slide had to be moved more often while drawing the cells than was necessary with normal motor cortex. Degenerating neurons and neuronophagic figures were rarely seen in the cerebral cortex.

Quantitative observations

The number of motor neurons per ventral horn (or hypoglossal nucleus) in a 20 μm section provided a simple quantitative measure of lower motor neuron loss. The number of motor neurons was, as expected, greatly reduced in the cases of ALS. The surviving motor neurons in ALS were smaller than normal: in the lumbar cord and in the hypoglossal nucleus, the mean cross-sectional area was only two-thirds of that of the motor neurons of the control subjects (*see* Table 2). In the spinal cord in ALS, there was a significant correlation ($r = 0.569$, $P < 0.05$), of the number of motor neurons with the cross-sectional areas of these cells: the neurons were smaller in the more severe cases of the disease. No such correlation could be found, however, in the hypoglossal nucleus.

TABLE 2 NUMBERS AND SIZES OF MOTOR NEURONS (HYPOGLOSSAL NUCLEI AND ANTERIOR HORNS OF SEGMENT L4) AND SIZES OF PYRAMIDAL CELLS IN LAYER 5 OF THE CORTEX OF THE PRECENTRAL GYRUS COMPARISON OF 10 CASES OF CLASSICAL SPORADIC AMYOTROPHIC LATERAL SCLEROSIS WITH 10 CONTROL SUBJECTS

	Subjects (mean ± SEM)		
Quantity measured	*Control*	*ALS*	*Significance of difference*
Hypoglossal neurons Number in 20 μm sections passing through each nucleus	39 ± 6	21 ± 9	$P < 0.0001$
Hypoglossal neurons Area (μm^2) in 20 μm sections passing through nucleolus	1055 ± 50	738 ± 85	$P < 0.005$
Cortical pyramidal neurons in tongue area Area (μm^2) in 20 μm sections passing through nucleolus, all cells	389 ± 35	320 ± 18	$P < 0.05$
Cortical pyramidal neurons in tongue area Area (μm^2) in 20 μm sections passing through nucleolus; largest 10% of cells measured	903 ± 59	744 ± 41	$P < 0.02$
Lumbar (L4) motor neurons Number per ventral horn in 20 μm sections through segment L4	46 ± 4	23 ± 3	$P < 0.0005$
Lumbar (L4) motor neurons Area (μm^2) in 20 μm sections passing through nucleolus	1278 ± 94	890 ± 103	$P < 0.01$
Cortical pyramidal neurons in foot/ankle area Area (μm^2) in 20 μm sections passing through nucleolus, all cells	516 ± 48	383 ± 36	$P < 0.02$
Cortical pyramidal neurons in foot/ankle area Area (μm^2) in 20 μm sections passing through nucleolus, largest 10% of cells measured	1825 ± 177	1021 ± 139	$P < 0.001$

No attempt was made to count neurons in the cerebral cortex, but the areas of sectioned pyramidal cells were measured in layer 5 of the parts of the precentral gyrus presumed to be concerned with movements of the foot and tongue. In the normal brains, the neurons were larger in the foot area (516 ± 48 μm^2) than in the tongue area (389 ± 35 μm^2). In both cortical regions, the mean neuronal area in the cases of ALS was approximately

75% of that in the controls (*see* Table 2). The range of size of normal cortical pyramidal cells was considerable. A few of these neurons were very large and conspicuous, thus qualifying as obvious Betz cells. However, there were also many moderately large neurons, and it was not possible to recognize the Betz cells as a distinct population. In order to detect pathological changes in the largest cortical neurons, samples comprising the largest 10% of measured cells from each specimen were extracted from the original data. As expected, these neurons were bigger in the foot area than in the tongue area, and the difference in size was larger than when pyramidal cells of all sizes were considered: $1825 \pm 177\ \mu m^2$ for the foot area, and $903 \pm 59\ \mu m^2$ for the tongue area, in the control subjects. In the foot area, the large neurons were 44% smaller in ALS than in the controls, whereas in the tongue area, the difference was only 17%. All the differences between measurements of neurons of control subjects and patients with ALS were statistically significant (Table 2).

The lower limbs were weak or paralysed to some extent in all patients with ALS, and there was corresponding depletion of motor neurons in segment L4. The surviving neurons in the lower lumbar cord and in the foot area of the precentral gyrus were smaller than those of control subjects (Table 2), but there was no significant positive or negative correlation of cortical neuronal size with either the numbers or the sizes of spinal motor neurons.

There was much variation in severity of paralysis of muscles supplied by the lower cranial nerves, and in 4 of the cases of ALS, there was little or no clinical evidence of paralysis of the tongue (Table 1). However, there was evidence of neuronal loss in the hypoglossal nucleus in all but one of these cases. The neurons in the tongue area of the primary motor cortex were smaller in ALS than in the control subjects (Table 2), but there was no significant correlation of cortical neuronal size with either the clinical score for severity of paralysis or with the number of motor neurons per sectioned hypoglossal nucleus (Table 3).

TABLE 3. CORRELATION OF CLINICAL SCORES, NUMBERS OF MOTOR NEURONS, AND AREAS OF MOTOR AND CORTICAL NEURONS, IN CASES OF AMYOTROPHIC LATERAL SCLEROSIS

Correlation of.	*With:*	*Correlation coefficient**
Clinical score for ankle and foot movements	Number of motor neurons per 20 μm section of ventral horn of L4	$r = +0.509$, $P < 0.05$
Clinical score for tongue movements	Number of motor neurons per 20 μm section of hypoglossal nucleus	$r = +0.138$, n.s.
Number of motor neurons per 20 μm section of ventral horn of L4	Mean area of sectioned motor neurons in L4	$r = +0.569$, $P < 0.05$
	Mean area of layer 5 pyramidal cells in foot area of precentral gyrus	$r = -0.154$, n.s.
	Mean area of largest 10% of measured cells in foot area	$r = -0.171$, n.s.
Number of motor neurons per 20 μm section of hypoglossal nucleus	Mean area of sectioned hypoglossal motor neurons	$r = +0.297$, n.s.
	Mean area of layer 5 pyramidal cells in tongue area of precentral gyrus	$r = +0.027$, n.s.
	Mean area of largest 10% of measured cells in tongue area	$r = -0.119$, n.s.

* P = probability that the correlation coefficient differs from zero (no correlation); n.s = not significantly different from zero.

DISCUSSION

The counts of cells in the ventral horn and hypoglossal nucleus in ALS provide numerical evidence of the loss of motor neurons that is the cardinal feature of this disease. Our findings agree closely with those of Tsukagoshi *et al.* (1979), who counted cervical (C6) motor neurons in 12 individuals diagnosed clinically as having ALS. The correlation of clinical scores for strength of movements of the ankles and feet with numbers of motor neurons in segment L4 (Table 3) was expected. However, there were some patients with no clinical evidence of paresis of the tongue in whom the hypoglossal neurons were depleted. This discrepancy might be due to inaccuracy of the clinical scoring method for lingual musculature, which is not as easy to assess as that of a limb. It is also possible that sprouting of intramuscular branches of intact hypoglossal axons can sometimes fully restore the strength of the tongue. Axonal sprouting is an important compensatory mechanism in partly denervated muscles (*see* Appel *et al.*, 1987) and can be detected electromyographically in the limbs in ALS (Brown, 1984; Appel *et al.*, 1987; Strong *et al.*, 1988).

Measurements of the areas of neurons in sections passing through the presumed centres of the cells showed that the surviving motor neurons in ALS were smaller than those of control subjects. This change might indicate that large neurons are the first to be lost, or that the cells shrink before they die. Although some chromatolytic neurons are seen in ALS, this change is quite different from that seen when the axons of healthy neurons have been severed. In the latter, the perikarya swell to larger than normal size (Barr and Hamilton, 1948). Reduced neuronal cross-sectional areas were also found in layer 5 of the primary motor area of the cerebral cortex in ALS. The largest cells in this layer, known as Betz cells, were conspicuously smaller than in the controls, though the change also affected smaller pyramidal cells (Table 2). Unlike the spinal cord and brainstem, the motor cortex in ALS contained hardly any obviously degenerating or necrotic neurons. Thus the smaller sizes were more likely to be due to shrinkage than to death and disappearance of only the larger cells.

Our suggestion of a transsynaptic mechanism of lower motor neuron degeneration (Hudson and Kiernan, 1988) was based on the lack of direct cortical projections to neurons that are regularly spared in ALS: those in the oculomotor, trochlear and abducent nuclei and in the nucleus of Onuf in segments S2-S3 of the spinal cord (Iwata *et al.*, 1978; Konno *et al.*, 1986; Holstege and Tan, 1987). However, the absence of any correlation between the severity of pathological change in the cortex and spinal cord (or brainstem) suggests that neuronal degeneration in these sites proceeds independently. The present results also fail to provide support for the idea that lower motor neuron degeneration is due to excessive responsiveness to a possibly toxic transmitter, such as glutamate (Plaitakis and Caroscio, 1987; Plaitakis *et al.*, 1988), secreted by corticospinal neurons. Neither is there morphometric evidence of retrograde transsynaptic degeneration of cortical neurons (Appel, 1981; Ugolini *et al.*, 1989).

The cell bodies of motor neurons shrink before they die in hereditary canine spinal muscular atrophy. This disease of Brittany spaniels resembles Werdnig-Hoffmann and Kugelberg-Welander diseases, which are hereditary motor neuron diseases of children. Cork *et al.* (1989) have shown that the muscles of affected dogs become weak and atrophic before their motor neurons die. The reduced sizes of the neurons may be associated with decreased synthesis and axonal transport of a myotrophic protein normally secreted at the neuromuscular junction (*see* Davis, 1988). Impaired axonal transport has been demonstrated in peripheral nerves of patients with ALS (Breuer *et al.*, 1987; Breuer and Atkinson, 1988).

Shrinkage of neurons has been recognized in other degenerative disorders of the nervous system including Alzheimer's and Parkinson's diseases (Terry *et al.*, 1981; Mann and Yates, 1983; Hansen *et al.*, 1988) and Huntington's disease (Oyanagi and Ikuta, 1987). It has been suggested (Mann and Yates, 1974; Mann, 1982) that the smaller perikarya are synthesizing reduced amounts of protein for transport into the axons. Indeed, convincing arguments have been made for the involvement of defective axonal transport of neuronotrophic agents in the pathogenesis of ALS and several other diseases in which interconnected sets of neurons degenerate (Gold, 1987; Saper *et al.*, 1987).

Karpati *et al.* (1988) have suggested that certain types of neuron, those that normally have conspicuous bundles of neurofilaments in their dendrites, are preferentially vulnerable in ALS. This suggestion could account for the loss of motor neurons, Betz cells and some other neurons such as those of the thoracic nucleus (Clarke's column) that often degenerate in ALS (Averback and Crocker, 1982; Hirano *et al.*, 1990; Williams *et al.*, 1990), and for the early changes seen in the dendrites of these cells (Hirano *et al.*, 1984). It does not explain the shrinkage of smaller neurons that we have detected in the motor cortex. However, there is little doubt that cell death in ALS is preceded by changes in the neurofilaments, which are chemically abnormal in naturally and experimentally diseased neurons (Silberberg and Kim, 1979; Shankar *et al.*, 1989).

The motor neurons (Chou, 1988; Kato *et al.*, 1989) and cortical pyramidal cells (Heggie *et al.*, 1989; Lowe *et al.*, 1989) in ALS also contain ubiquitin, a protein that binds to cytoskeletal filaments and renders them susceptible to attack by ATP-dependent proteolytic enzymes. Ubiquitin does not occur in healthy nervous tissue, but in ALS it is immunohistochemically demonstrable in eosinophilic cytoplasmic inclusions (Bunina and Lewy-like bodies) and, much more abundantly, as globose and skein-like accumulations not recognizable in conventionally stained sections. Ubiquitination of abnormal neurofilament proteins has also been observed in Alzheimer's, Pick's and Parkinson's diseases (Lee *et al.*, 1988; Leigh *et al.*, 1988). As in ALS, the protein is present in recognized inclusion bodies and also in neurons that are not otherwise visibly abnormal. In various diseases, therefore, the presence of ubiquitin is a sensitive indicator of cells that are destined to degenerate (Gallo and Anderton, 1989). It seems likely that proteolytic degradation of cytoskeletal proteins would lead to reduction in the sizes of the affected neuronal perikarya.

ACKNOWLEDGEMENTS

The technical assistance of Mr Alastair Todd and Miss Karen Chapman is gratefully acknowledged. This work was supported by a grant from the Amyotrophic Lateral Sclerosis Society of Canada.

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(Received February 2, 1990. Revised May 16, 1990. Accepted May 22, 1990)

Brain (1991), **114**, 855–866

HEREDITARY ATAXIAS AND PARAPLEGIAS IN CANTABRIA, SPAIN

AN EPIDEMIOLOGICAL AND CLINICAL STUDY

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SUMMARY

A clinical, genetic and epidemiological study of hereditary ataxias and paraplegias was conducted within a defined area (Cantabria) in Northern Spain from 1974 to 1986. The series comprised 48 index cases and 65 affected relatives. On prevalence day, 103 patients were alive, giving a prevalence of 20.2 cases per 100 000. There were 24 patients (18 families) with Friedreich's ataxia (FA), 12 (6 families) with early onset cerebellar ataxia (EOCA) differing from FA, 6 (3 families) with dominantly transmitted late onset cerebellar ataxia (LOCA), 11 with 'idiopathic' LOCA, 49 (9 families) with 'pure' hereditary spastic paraplegia (HSP), and 1 patient with congenital cerebellar ataxia. The prevalence found here is comparable with the highest figures described in previous surveys. This may in part be due to the great number of secondary cases in our series. A high frequency of parental consanguinity occurred in FA patients, 'pseudodominant' inheritance being observed in 1 family. The clinical features were those of classical FA except for later onset and slower course in 1 family, and retained tendon reflexes in the lower limbs in 2 cases. Such data indicate the need for modification of the essential criteria for the disease. EOCA included 4 patients with normoreflexic ataxia and 1 patient with ataxia and luteinizing hormone-releasing hormone deficiency. In addition, there were 7 patients from 2 unrelated families with a homogeneous syndrome characterized by autosomal recessive inheritance, cerebellar ataxia, retinitis pigmentosa and sensory neuropathy. This syndrome is therefore a well defined nosological entity to be added to the list of autosomal recessive mendelian phenotypes. The clinical picture of patients with LOCA was either a 'pure' cerebellar or a 'cerebellar-plus' syndrome. Genetic subgroups of 'pure' HSP were autosomal dominant type I in 5 families and type II in 2, and autosomal recessive in 2 families.

INTRODUCTION

Hereditary ataxias and paraplegias encompass a heterogeneous group of disorders of unknown cause, the outstanding characteristics of which are ataxia or spastic paraplegia and spinocerebellar atrophy. Due to their complex nosology there have been many attempts at classification. As stated by Refsum and Skre (1978) 'it is not any great exaggeration to say that there are as many proposed classifications as there are authors on the subject'. Classifications have usually been based on pathological and clinical features (Holmes, 1907; Greenfield, 1954; Konigsmark and Weiner, 1970). These are unsatisfactory because they ignore the genetic heterogeneity both of the clinical picture and pathological framework (Harding, 1982; Berciano, 1988). Recently, Harding (1983, 1984) proposed an accurate classification based on clinical and genetic features that

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has gained worldwide acceptance, and which allows clinicians to diagnose the majority of patients. This is an essential step for epidemiological purposes (Schoenberg, 1978*a*).

Prevalence data for hereditary ataxias and paraplegias are scanty (Bobowick and Brody, 1975) and most studies either departed from clinicopathological classifications or were focused on specific syndromes. The aims of the present study were to screen a limited geographical area in Northern Spain to establish figures for the prevalence and relative frequency of genetic and clinical subtypes of hereditary ataxias and paraplegias.

PATIENTS AND METHODS

The study was performed in the Autonomous Community of Cantabria, which lies in Northern Spain and covers an area of 5289 km^2 with a population of 510 000 inhabitants (1981 census).

The patients were referred to the National Hospital 'Marqués de Valdecilla' between 1974 and 1986. This is the main hospital to which all neurological problems are referred. The 4 private hospitals in the region treat only acute medical and surgical problems and there are no consultant neurologists other than those at the hospital. A recent survey has estimated that more than 90% of people always use the public health service (according to the 1988 official statistics of the Spanish Sociological Investigations Centre). Thus the neurological patient population in this hospital is truly representative of the region of Cantabria.

Proband patients were initially evaluated at the hospital. Family trees were routinely performed and cooperative individuals at risk were visited in hospital or at home. Serial clinical examinations, yearly or less, were regularly carried out. Using Harding's (1983, 1984) classification the patients were divided into six groups: congenital cerebellar ataxia; Friedreich's ataxia (FA); early onset cerebellar ataxia (EOCA) differing from FA; late onset cerebellar ataxia (LOCA), autosomal dominant and 'idiopathic'; and hereditary spastic paraplegia (HSP). HSP was accepted here when together with the index case there was objective evidence of disease in at least 1 additional member of the family. Routine haematological and biochemical studies, radiographs of the skull, syphilitic serology, electrocardiography, motor and sensory nerve conduction studies of median and/or peroneal nerves, somatosensory and auditory evoked potentials, electromyography of tibialis anterior and/or abductor pollicis brevis muscles were performed in each index patient and in most secondary cases. Most patients with hereditary ataxia also had an EEG and cranial CT scan. The majority of FA patients also had an echocardiogram. Specific tests for detecting hexosaminidase deficiency, juvenile Niemann-Pick disease and metachromatic leucodystrophy were performed in selected patients.

Patients were considered to be resident if they were residing in Cantabria in 1986. All prevalence rates will be here referred to as point prevalence (Schoenberg, 1978*b*) on the last day of 1986 Since we routinely examined most subjects at risk in each pedigree, virtually all secondary cases were detected and therefore there was only 1 proband per family.

RESULTS

During the period of study, 76 index cases were considered to have hereditary ataxia or spastic paraplegia; 28 cases were excluded (Table 1). Consequently 48 propositi entered finally for epidemiological analysis. Moreover, 178 relatives were examined of whom 51 were found to be affected. A further 14 relatives were known to be affected by history. Ten patients died before the prevalence day. Thus there were 103 patients living on 31 December 1986. The total point prevalence was 20.2 cases per 100 000 population. Prevalence rates for the different subtypes are summarized in Table 2.

Congenital cerebellar ataxia

Congenital cerebellar ataxia was diagnosed only in 1 patient born of a normal pregnancy. Parental consanguinity was not recognized. He had started walking at 2 yrs.

TABLE 1 EXCLUDED CASES

Not resident in Cantabria		10
Other diagnoses		12
Alcoholic cerebellar atrophy (4)		
Parkinsonism (2)		
Phenytoin intoxication (1)		
Hallervorden-Spatz disease (1)		
Niemann-Pick disease type C (1)		
Multiple sclerosis (1)		
Amyotrophic lateral sclerosis (1)		
Metachromatic leucodystrophy (1)		
Incomplete study		6
	Total	28

TABLE 2 CASE DISTRIBUTION INTO SUBTYPES

Subtype	*Probands*	*Secondary cases*	*Living 31.12 1986*	*Prevalence per 100 000*
Congenital ataxia	1	–	1	0.2
Friedreich's ataxia (FA)	18	7	24	4.7
EOCA differing from FA	6	7	12	2.3
Autosomal dominant LOCA	3	5	6	1.2
'Idiopathic' LOCA	11	–	11	2.2
'Pure' hereditary spastic paraplegia	9	46	49	9.6
Total	48	65	103	20.2*

EOCA = early onset cerebellar ataxia; LOCA = late onset cerebellar ataxia * 95% confidence interval, 16.4–24.3.

Afterwards he manifested gait difficulty, but this was not apparently progressive. On examination at age 17, there was pes cavus, static and kinetic cerebellar ataxia, dysarthria, bilateral impairment of lateral gaze, nystagmus, distal hypopallaesthesia, and extensor plantar responses. Mental status was normal. There was neither optic atrophy nor hypoacusis. Motor and nerve conduction velocities of median and peroneal nerves were normal. CT scan revealed brainstem and vermal atrophy.

Friedreich's ataxia

Leaving aside a case affected by history, the clinical characteristics of 24 FA patients were as follows. There were 10 males and 14 females. There was parental consanguinity in 9 families (50%), 'pseudodominant' inheritance being present in 1 (fig. 1). The mean age of onset was 13.08±8.37 (range 3–35) yrs. The mean age at the time of study was 25.58±13.51 (range 7–56) yrs, and the average duration of the disease was 12.50±9.63 yrs. Two patients, brother and sister, with onset at 30 and 35 yrs and relatively slow clinical course, belonged to the family with 'pseudodominant' transmission which was included in the genetic study that provided evidence of genetic homogeneity at the FA locus on chromosome 9 (Chamberlain *et al.*, 1989).

Initial symptoms included gait disturbances in 24 (100%), foot deformities in 5 (21%), manual clumsiness in 4 (17%), dysarthria in 4 (17%) and dysphagia in 1 (4%). The

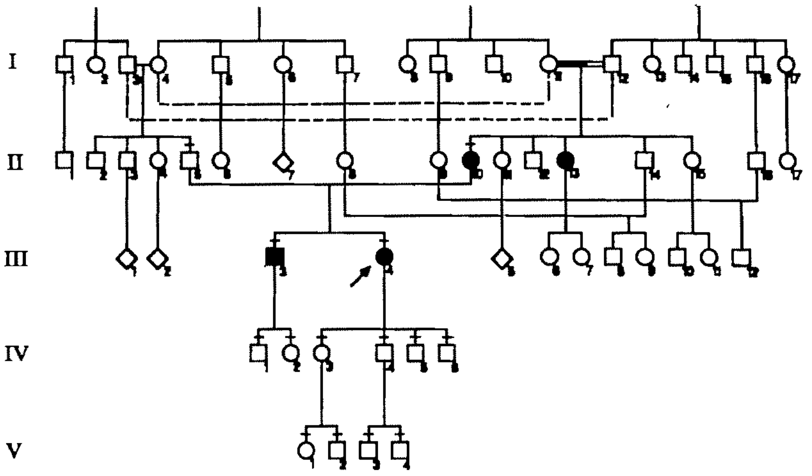

FIG. 1. Pedigree of family with FA and 'pseudodominant' inheritance. Broken lines indicate consanguinity. Symbols with horizontal marks correspond to examined patients. Case II-10 was examined in 1988 and consequently not included in this study.

clinical picture is summarized in Table 3. All patients had had progressive ataxia. Knee jerks and ankle jerks were preserved in 2 patients, 10 and 15 yrs after onset. One of these was an index case who fulfilled all other essential and additional criteria of FA (Harding, 1984); the other corresponds to Case III-3 in fig. 1. Two patients had associated congenital glaucoma (Combarros *et al.*, 1988) and were also included in the genetic study reported by Chamberlain *et al.* (1989). All probands had evidence of the characteristic ECG abnormalities as did 4 out of 6 secondary cases. Serial ECGs in 1 patient revealed the appearance of T wave inversion. Echocardiography showed abnormalities in 13 out of 19 (68%) cases. Only 3 patients had cardiac symptoms consisting of atrial fibrillation with heart failure and systemic embolism (2 cases), and angina (1 case). Cardiac disease caused death in 1 patient (Table 2), postmortem examination revealing the characteristic findings of the disease (Hughes *et al.*, 1968;

TABLE 3. FRIEDREICH'S ATAXIA* ESTABLISHED CLINICAL PICTURE

	No. of cases (%)
Cerebellar ataxia	24 (100)
Distal hypopallaesthesia	24 (100)
Dysarthria	22 (92)
Absent knee and ankle jerks	22 (92)
Scoliosis	18 (75)
Pes cavus	18 (75)
Pyramidal weakness in lower limbs	17 (71)
Extensor plantar responses	15 (62)
Nystagmus	8 (33)
Optic atrophy	2 (8)
Congenital glaucoma	2 (8)

*(n = 24)

Lamarche *et al.*, 1984; Oppenheimer, 1984). Two (8%) cases had diabetes controlled with insulin and oral hypoglycaemic drugs.

Nerve conduction studies were carried out in all 24 patients. Motor conduction velocities in the median nerve ranged from 46 to 69 $m \cdot s^{-1}$ (54.4 ± 7.6). Distal sensory potentials of median nerve were absent or reduced (between 0.5 and 3 μV). A CT scan was performed in 22 cases. As previously described by us (Ramos *et al.*, 1987), the scan either revealed no abnormalities in the posterior fossa (10 patients) or moderate cerebellar atrophy with an increase in the surface area of the fourth ventricle (12 patients).

EOCA differing from FA

This group includes 12 patients belonging to 6 families. The diagnostic categories were as follows: ataxia, retinitis pigmentosa and sensory neuropathy (7 patients in 2 families), ataxia with retained tendon reflexes (4 patients in 3 families) and ataxia with luteinizing hormone-releasing hormone (LHRH) deficiency (2 patients in 1 family).

Inheritance in the 2 families with ataxia, retinitis pigmentosa and sensory neuropathy was either autosomal recessive or 'pseudodominant' (fig. 2). Six patients were examined and 1 was affected by history. The age of onset ranged from 2 to 14 yrs. Initial symptoms in the 6 examined patients were gait disturbances in 4 and loss of vision in 2. Age at examination ranged from 4 to 40 (median 22.5) yrs. The clinical picture was homogeneous and included cerebellar ataxia, dysarthria, generalized areflexia, marked distal hypopallaesthesia, and diffuse pigmentary retinopathy with severe loss of visual acuity and tubular vision. In no case was there evidence of ophthalmoplegia, amyotrophy, foot deformities, pyramidal signs, deafness, nystagmus, mental deterioration or ichthyosis. All patients were able to walk without assistance. ECG as well as routine laboratory investigations including betalipoproteins were consistently normal. Nerve conduction studies on the median and/or peroneal nerves, performed in 4 cases, revealed absence of distal sensory potentials with normal motor conduction. In 3 cases CT scans did not reveal abnormalities. Serum phytanic acid levels were not available.

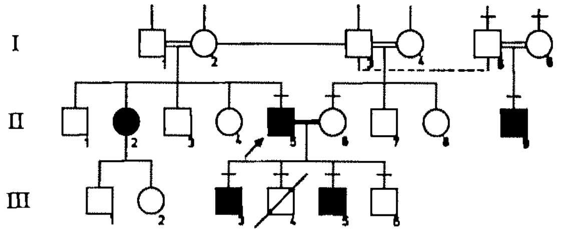

FIG. 2. Pedigree of family with cerebellar ataxia, retinitis pigmentosa and sensory neuropathy, and 'pseudodominant' inheritance For symbols, *see* fig. 1

In the ataxia with retained tendon reflexes group there were 3 males and 1 female. Parental consanguinity was present in 1 case, but genealogical data were insufficient to determine the pattern of transmission. The onset of symptoms ranged from 6 to 22 yrs.

The clinical picture was essentially that of moderate spastic ataxia. There was no evidence of cardiomyopathy. Motor and sensory conduction velocities in the median or peroneal nerves were normal. CT scan revealed cerebellar atrophy with normal brainstem parameters.

The characteristics of the family with ataxia and LHRH deficiency have been reported previously (Berciano *et al.*, 1982). The proband died during the period of study (Table 2). Unfortunately, postmortem examination was not performed.

Autosomal dominant LOCA

There were 8 patients belonging to 3 families with autosomal dominant ataxia. Five patients (2 families) had 'cerebellar-plus' syndrome, consisting of gait and limb ataxia, dysarthria, pyramidal and spinal signs, supranuclear ophthalmoplegia, dysphagia, sphincter disturbances or dementia. Two patients showed parkinsonian signs. Ages at onset ranged from 12 to 40 (median 28) yrs. Pneumoencephalography (1 case) and CT scan (3 cases) revealed severe cerebellar and brainstem atrophy and an increase in the size of the fourth ventricle (Berciano *et al.*, 1983; Ramos *et al.*, 1987). Two sisters died during the period of study, postmortem examination in 1 case revealing olivopontocerebellar atrophy (OPCA) with spinal involvement (Berciano *et al.*, 1983).

Three patients, from a family in which the proband and 2 affected cousins were living, had a 'pure' cerebellar syndrome of later onset, this developing at 55 yrs in the only examined patient. A CT scan in this case revealed cerebellar vermis and hemisphere atrophy with normal brainstem parameters.

'Idiopathic' LOCA

This group includes 11 patients (8 males, 3 females). The mean age of onset was 47.4 ± 15.3 (range 28–70) yrs. Six patients had a 'cerebellar-plus' syndrome consisting of cerebellar ataxia together with pyramidal signs, dementia, supranuclear ophthalmoplegia, dysphagia, sphincter disturbances, hypopallaesthesia or chorea. None of these cases showed parkinsonian signs. Five patients had a 'pure' cerebellar syndrome. The duration of the disease at the time of study in these cases ranged from 11 to 19 yrs. CT scan findings were comparable with those in the previous group.

'Pure' hereditary spastic paraplegia

There were 9 index cases; moreover, 109 relatives were examined, of whom 36 were found to be affected. A further 10 relatives were affected by history. Inheritance was autosomal dominant in 7 families and recessive in 2. According to the age of onset of dominant cases, 5 families corresponded to type I (onset below 35 yrs) and 2 to type II (onset over 35 yrs) (Harding, 1981*a*). Genetic, clinical and electrophysiological details of these families will be published later.

DISCUSSION

Since 1923, 13 epidemiological studies on hereditary ataxias and paraplegias have been reported, which are summarized in Table 4. Furthermore, on the basis of the estimates of disease duration, the prevalence rate of hereditary ataxias and paraplegias between 10 and 50 yrs of age was 5.7 cases per 100 000 in the island of Zealand

TABLE 4 SUMMARY OF PREVALENCE RATES OF HEREDITARY ATAXIAS AND PARAPLEGIAS

Author	*Area of survey*	*Time period*	*Population*	*No. of cases*	*Prevalence per 100 000*
Hanhart (1923)	Switzerland	1921	3 500 000	46	3.7[1]
Sjogren (1943)	Sweden	1905–1941	6 000 000	120	2.0
Kurland (1958)	Rochester, Minnesota	1945–1954	29 885	0	0 0
Kreindler *et al.* (1964)	Rumania	1950–1962	?	395	2 0[2]
Brewis *et al* (1966)	Carlisle	1955–1961	71 101	5	7 0
Chen *et al* (1968)	Guam	1960–1966	37 975	9	23.7
Gudmundsson (1969)	Iceland	1954–1963	187 200	2	1.1
Koeppen *et al.* (1977)	Scotland	?	5 228 963	16[3]	0.3
Trizio *et al.* (1980)	Puglia (Italy)	?	?	139	3.5
Skre (1980)[4]	Western Norway	1960–1968	725 000	68	22 1[5]
Lucci *et al.* (1982)	Reggio-Emilia (Italy)	1965–1980	412 324	21	4.2
Sridharan *et al.* (1985)	Benghazi (Libya)	1982–1984	519 000	25	4.8
Brignolio *et al* (1986)	Torino (Italy)	1945–1982	2 327 996	142	6 1
Present series	Cantabria	1974–1986	510 000	103	20.2

[1] Extrapolated estimate. [2] Average from 20 cities and territories (prevalences ranging from 0.21 to 4 27). [3] Only adult onset hereditary ataxia. [4] Adjusted in relationship to earlier figures. [5] Prevalence corrected by estimation of ascertainment probability.

(Werdelin, 1986). Since diagnostic criteria, methodology of study and classification of hereditary ataxias have been a changing matter, comparison of our data with previous studies should only be made with caution. In any case, the prevalence found in Cantabria, 20.2/100 000, is comparable with the highest prevalence figures described in other parts of the world (Chen *et al.*, 1968; Skre, 1980). Although in our study we had a greater number of secondary cases, prevalence rate remains high even when only index cases are considered, 9.4/100 000. As described in Patients and Methods, the neurological patient population in our hospital is truly representative of that of Cantabria. Parallel epidemiological data have been obtained for multiple sclerosis, Charcot-Marie-Tooth disorders and motor neuron disease (Miró *et al.*, 1984; Combarros *et al.*, 1987; López-Vega *et al.*, 1988).

Congenital cerebellar ataxia had not been considered in previous studies. In this series there was only 1 patient, thus representing a prevalence of 0.2. From the CT scan findings (Ramos *et al.*, 1987), the presumptive diagnosis here was olivopontocerebellar hypoplasia.

Taking into account the number of probands, FA was the most common disorder in this series (Harding, 1984). Previous surveys are summarized in Table 5. Moreover, Werdelin (1986) reported a prevalence rate of 0.5 for males and 1.2 for females among individuals aged 10–50 yrs. FA prevalence in Cantabria is clearly higher than previous estimations, with the exception of the genetic isolate in Kathikas-Arodes (Cyprus) where 13 FA patients were found in a population of 709 (Dean *et al.*, 1988). Intriguingly, however, the proportion of FA families in our study and in Harding's case series (Harding, 1982) are very similar, that is, 0.37 as opposed to 0.45. The high consanguinity rate in our patients has also been described in other FA series (Skre, 1975; Ülkü *et al.*, 1988). Unfortunately, there are no official data about the frequency of consanguinous marriages in the general population in Cantabria; consequently we did not try to calculate the gene frequency of the disease. 'Pseudodominant' inheritance (Harding and Zilkha,

TABLE 5. SUMMARY OF PREVALENCE RATES OF FRIEDREICH'S ATAXIA

Author	*Area of survey*	*Time period*	*Population*	*No. of cases*	*Prevalence per 100 000*
Sjögren (1943)	Sweden	1905–1941	6 000 000	73[1]	1.2
Kurland (1958)	Rochester, Minnesota	1945–1954	29 885	0	0.0
Chen *et al.* (1968)	Guam	1960–1966	37 975	0	0.0
Gudmundsson (1969)	Iceland	1954–1963	187 200	2	1.1
Skre (1975)[2]	Western Norway	1960–1968	725 000	5	1.0[3]
Trizio *et al.* (1980)	Puglia (Italy)	?	?	29	0.7
Filla and Campanella (1980)	Campania (Italy)	1969–1978	?	53	1.1
Lucci *et al.* (1982)	Reggio-Emilia (Italy)	1965–1980	412 324	6	1.4
Brignolio *et al.* (1986)	Torino (Italy)	1945–1982	2 327 996	23	1.0
Present series	Cantabria	1974–1986	510 000	24	4.7

[1] Only typical FA according to the author. [2] Adjusted in relationship to earlier figures (*cf* Skre, 1980). [3] Prevalence corrected by estimation of ascertainment probability.

1981) was observed in 1 of our families with obvious parental consanguinity and affected members in 2 generations.

Clinical and electrophysiological features in our FA patients differed little from previous reports (Tyrer, 1975; Geoffroy *et al.*, 1976;. Harding, 1981*b*, 1984). Two families deserve comment. In the family with 'pseudodominant' transmission the ages of onset were somewhat later than usual, just as occurred in the family described by Harding and Zilkha (1981), and patients exhibited a much slower course and less severe clinical picture (Barbeau *et al.*, 1984; Chamberlain *et al.*, 1989; Keats *et al.*, 1989). Retained tendon reflexes in the lower limbs was observed in 2 patients. One belonged to the family with the 'pseudodominant' inheritance just mentioned and the other otherwise had the clinical picture of classical FA. These data indicate a need for modification of current diagnostic criteria in relation to lower limb areflexia (Martin *et al.*, 1988) and the age of onset of the disease.

As a whole, the group of patients with EOCA differing from FA represents 10% of our series. There were three main diagnostic categories. Four patients belonging to 3 families had ataxia with retained tendon reflexes. As characteristic of this entity, inheritance was most probably autosomal recessive, none of the patients showed cardiomyopathy, skeletal deformities, optic atrophy or diabetes mellitus, the clinical course was much slower than in FA, and sensory nerve conduction velocities were normal (Harding, 1981*c*; Özeren *et al.*, 1989). The clinical findings in our 2 families with ataxia, retinitis pigmentosa and sensory neuropathy are comparable. Transmission was either autosomal recessive or 'pseudodominant'. The clinical picture included loss of vision, and cerebellar and tabetic ataxia. Electrophysiologically the only finding was absence of distal sensory nerve action potentials. None of the patients had electrocardiographic evidence of heart block or ophthalmoplegia to suggest the Kearns-Sayre syndrome, but we have to admit that other types of mitochondrial disease have not been excluded with certainty. Although we did not determine serum phytanic acid levels, the absence of many signs usually present in Refsum's disease and the selective impairment of sensory nerve conduction allowed us to exclude this diagnosis (Refsum, 1984). Autosomal dominant cerebellar ataxia with pigmentary retinal degeneration is a clinically distinct syndrome (Harding, 1982). Autosomal recessive phenotypes are heterogeneous and as well as cerebellar and visual symptoms, there is a variable combination of mental retardation, dementia, pyramidal signs, scoliosis and distal amyotrophy (*cf* Harding, 1984). The clinical picture in our patients, on the contrary, was homogeneous and almost identical to Case 2 of Tuck and McLeod (1983), indicating that the syndrome represents a well defined nosological entity to be included among autosomal recessive mendelian phenotypes (McKusick, 1988). Finally, there was in this group a family with ataxia and hypogonadotrophic hypogonadism. Although in this family the hypogonadism was probably due to LHRH deficiency (Berciano *et al.*, 1982), the same phenotype has recently been related to a more widespread endocrine disturbance involving both the hypothalamus and pituitary (Fok *et al.*, 1989).

The proportion of patients with LOCA in this series was 0.29, which coincides again with the estimation of 0.23 in Harding's series (Harding, 1982). In two recent studies on dominantly inherited OPCA, prevalences of 41/100 000 in the province of Holguin (Cuba) (Orozco *et al.*, 1989) and 13–184/100 000 in the Iakut population of Eastern Siberia (Goldfarb *et al.*, 1989) were found. These extremely high prevalences are probably due to a 'founder effect' (Orozco *et al.*, 1989). In our patients there were two basic clinical patterns: a 'pure' cerebellar syndrome in 42% of cases, and a 'cerebellar-plus' syndrome in 58% (Harding, 1982). According to pneumoencephalographic or CT scan findings (Ramos *et al.*, 1987), in the first pattern the presumptive pathological diagnosis was cortical cerebellar atrophy, and OPCA in the second. This pathological label was confirmed in 1 case (Berciano *et al.*, 1983). It is worthwhile noting that only 2 patients with OPCA had had parkinsonian features, a fact that militates against the statement that extrapyramidal dysfunction is an essential component of the disease (Duvoisin, 1987).

Finally, prevalence rates of 'pure' HSP are summarized in Table 6. Werdelin (1986) reported a prevalence of 1.1/100 000. Considering that in the Guam survey 4 sporadic cases were included and that the crude prevalence rate in Skre's study was 4.7/100 000, the prevalence found in Cantabria, 9.6/100 000, is again higher than previous estimations. The large number of secondary cases in our series justifies this apparent discrepancy.

TABLE 6. SUMMARY OF PREVALENCE RATES OF 'PURE' HEREDITARY SPASTIC PARAPLEGIA

Author	*Area of survey*	*Time period*	*Population*	*No. of cases*	*Prevalence per 100 000*
Chen *et al* (1968)	Guam	1960–1966	37 975	7	18.4
Skre (1980)[1]	Western Norway	1960–1968	725 000	31	11.9[2]
Lucca *et al.* (1982)	Reggio-Emilia (Italy)	1965–1980	412 324	4	1.0
Brignolio *et al* (1986)	Torino (Italy)	1945–1982	2 327 996	31	1.3
Present series	Cantabria	1974–1986	510 000	49	9.6

[1] Adjusted in relationship to earlier figures [2] Prevalence corrected by estimation of ascertainment probability.

ACKNOWLEDGEMENTS

The authors are very grateful to Stella Camenta for translation of Kreindler's paper and to John Hawkins for stylistic revision of the manuscript. The study was supported in part by Fondo de Investigaciones Sanitarias, grant no. 88/1502.

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(*Received March 13, 1990. Revised May 22, 1990. Accepted May 29, 1990*)

Brain (1991), **114**, 867–892

SURAL NERVE MORPHOMETRY IN DIABETIC AUTONOMIC AND PAINFUL SENSORY NEUROPATHY

A CLINICOPATHOLOGICAL STUDY

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SUMMARY

Observations have been made on a selected series of insulin-dependent patients with neuropathy, subdivided into three groups: (1) severe autonomic neuropathy with an accompanying painless sensory neuropathy; (2) severe autonomic neuropathy with a chronic painful sensory neuropathy; and (3) chronic or acute painful sensory neuropathy with no autonomic neuropathy. All three groups showed a loss of large and small myelinated nerve fibres in sural nerve biopsy specimens which was greater in Groups 1 and 2. Regenerative activity was prominent in all three groups, but least in Group 3. Teased fibre studies showed evidence both of axonal regeneration and remyelination. Active fibre degeneration was rare. Measurements of g ratio (axon diameter:total fibre diameter) gave no indication of axonal atrophy. The density of unmyelinated axons was reduced in all three groups, as was their median diameter.

Vibration sense threshold was positively correlated with the total number of myelinated fibres and thermal sensory threshold with median unmyelinated axon diameter but not with total unmyelinated axon numbers. No correlation between the occurrence of pain and active degeneration of myelinated fibres or with regenerative activity either in myelinated or unmyelinated axons was detectable. Assessment of differential loss of large or small myelinated nerve fibres was difficult because of the presence of large numbers of small regenerating myelinated axons.

The results are discussed in relation to the concept of 'diabetic small fibre neuropathy' and the causation of pain in diabetic neuropathy.

INTRODUCTION

The disturbances of peripheral nerve function associated with diabetes mellitus are highly diverse, as witnessed by the variety of classifications that have been proposed (*see* Thomas and Brown, 1987). The commonest manifestation is a generalized and approximately symmetric polyneuropathy that predominantly involves sensory and autonomic function. Within this category, subtypes have been defined, such as painful 'small fibre' neuropathy (Brown *et al.*, 1976), acute painful diabetic neuropathy (Archer *et al.*, 1983) and cases

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with recurrent foot ulceration (Young *et al.*, 1986). It has been argued as to whether this reflects the operation of multiple causative mechanisms (Thomas and Eliasson, 1984) or whether there is a single major aetiological process with modifying factors.

There is debate as to the nature of the pathological changes that underlie the generalized sensory and autonomic polyneuropathy. One proposal is that it represents a distal length-related neuropathy of 'dying-back type' (Schaumburg *et al.*, 1983), with the implication that it could have a metabolic basis. The second is that it is the consequence of the summation of multifocal lesions in the peripheral nervous system (Dyck *et al.*, 1986*a, b*) probably on an ischaemic basis. When subgroups with the sensory polyneuropathy category are considered, does a patient with a painful small fibre neuropathy fall into a distinct subgroup as suggested by Brown *et al.* (1976) or is this merely an artificial separation from a continuous spectrum of pathological variables (Dyck *et al.*, 1986*b*)? If such syndromes constitute separate subtypes, this would have aetiological implications in indicating the selective vulnerability of particular sets of neurons or the operation of multiple causative mechanisms.

The correlation between morphological parameters and symptoms has given rise to discussion, for example in relation to painful diabetic neuropathy. Regenerated axonal sprouts from small myelinated and unmyelinated axons have been suggested as being involved (Asbury and Fields, 1984; Llewelyn *et al.*, 1986). In a range of neuropathies, Dyck *et al.* (1976) correlated the occurrence of 'painfulness' with the presence of acute fibre breakdown in sural nerve biopsies.

It has been established that nearly all patients with clinical symptoms of autonomic dysfunction will also have an associated somatic neuropathy (Tackmann *et al.*, 1981). The relationship between patients with sensory polyneuropathy and autonomic dysfunction is less distinct (Young *et al.*, 1986). The morphological findings in peripheral nerve biopsies in patients with severe autonomic involvement have not yet been established.

In this study, we have assessed morphometric changes in sural nerve biopsies in patients with severe autonomic neuropathy (Group 1). Some patients also had pain as a prominent symptom (Group 2). Other patients had an acute or chronic painful neuropathy with no symptoms of autonomic dysfunction (Group 3).

PATIENTS

Nineteen insulin-dependent diabetic patients (4 male, 15 female; age range 18–54 yrs) were selected for this study because of the presence of pain and/or autonomic neuropathy with or without iritis. All had a symmetric distal neuropathy. Clinical symptoms, nerve conduction studies, or temperature and vibration threshold assessment confirmed the presence of neuropathy. Autonomic symptoms were defined as follows: *diabetic diarrhoea*—intermittent, mainly nocturnal diarrhoea, usually with faecal incontinence and no evidence of other gastrointestinal disorder; *gustatory sweating*—facial sweating precipitated by eating specific foods; *postural hypotension*—hypotensive symptoms on standing associated with a drop in systolic blood pressure of 30 mmHg or more; *gastroparesis*—recurrent vomiting and normal gastroscopy, with residual fluid and food in the stomach after an overnight fast and delayed transit of barium either on i.v. urography or bladder ultrasound studies with no mechanical outflow obstruction. Autonomic function was assessed from the clinical symptoms and by measurement of heart rate variability, postural change in pulse rate and blood pressure and heart rate response to the Valsalva manoeuvre expressed as the Valsalva ratio (*see below*).

Clinical details and biochemical data at the time of biopsy are given in Tables 1, 2, 3. There was no evidence of focal neuropathy and other possible causes of neuropathy were excluded. None of the patients had symptoms of peripheral vascular disease, but 2 (Cases 14, 19) had absent foot pulses.

Informed consent was obtained prior to sural nerve fascicular biopsy, with the approval of the Ethics Committee at King's College Hospital. Normal control sural nerve was obtained from 5 organ donor cases (1 male, 4 female; age range 28–55 yrs) before or, in one instance, within 45 min of cessation of ventilation, following permission from the next of kin and with the approval of the Ethics Committee at the Royal Free Hospital.

METHODS

Clinical

Neurophysiology. Sensory action potentials and conduction velocities were measured in the median, radial and sural nerves using surface electrodes. For motor studies, the terminal latencies, compound muscle action potentials, conduction velocities and F wave latencies were obtained for the median and tibial nerves, recording from abductor pollicis brevis and abductor hallucis, respectively. Distal limb temperature was raised to between 32 and 34° C when required and recordings were made using either a Medelec MS6 (Medelec, Woking, Surrey, UK) or a Dantec Neuromatic (Dantec, Bristol, UK) electromyograph.

Temperature and vibration threshold. Temperature and vibration thresholds were recorded in the dominant limb at a constant room temperature (20° C). Using a Marstock device (Somedic, Stockholm, Sweden), temperature sensation was measured on the thenar eminence of the hand and over the site of extensor digitorum brevis on the dorsum of the foot. The warm-cool difference was expressed in degrees Celsius. Vibration sensation was measured over the pulp of the index finger and hallux using a hand-held biothesiometer (Biomedical Instruments, Newbury, Ohio, USA) and expressed in volts. Values for normal control subjects have previously been reported from the Diabetic Department, King's College Hospital (Guy *et al.*, 1985).

Autonomic function tests. Autonomic function tests were performed with the patient in a semirecumbent position in a quiet room (temperature 21–23° C) and following a period of rest for 15 min before testing. A Lectromed (St Ouen, Jersey, Channel Islands) instantaneous heart rate meter was used to measure heart rate variation on deep breathing at 6 breaths per min (MacKay *et al.*, 1980), heart rate increase on standing, and the response to a standard Valsalva manoeuvre (Ewing and Clarke, 1982). Blood pressure change on standing was measured using a standard mercury sphygmomanometer.

Histological processing

Tissue source. Under local anaesthesia with 1% lignocaine, sural fascicular nerve biopsies were obtained from a site immediately posterior to the lateral malleolus. Total nerve biopsies were not performed to avoid producing further sensory loss and the risk of 'trophic' ulceration.

Tissue preparation. After initial fixation of the specimens in 3% glutaraldehyde in PIPES (piperazine-n,n'-bis [2-ethane-sulphonic acid]) buffer (Baur and Stacey, 1977) with 2% sucrose, the tissues were postfixed in 1% osmium tetroxide in PIPES buffer with 1.5% potassium ferricyanide and later dehydrated through increasing concentrations of ethyl alcohol and processed via 1-2 epoxypropane, before being embedded in Araldite. Material for the isolation of single fibres was fixed as above but processed through toluene and stored in Araldite without accelerator.

Morphometric studies. Transverse semithin sections (1 μm) of the embedded material were cut and stained with thionin and acridine orange (Sievers, 1971). Using a ×40 objective on a Leitz optical microscope connected on-line through a television camera to a Kontron MOP Videoplan image analyser, fascicular area, myelinated fibre (MF) density and size distribution were estimated. Unmyelinated axon (UMA) density and size distribution were determined from electron micrographs (JEOL 100CX electron microscope) of thin sections stained with uranyl acetate and lead citrate. In combination with staining in block with potassium ferricyanide, this produces a denser staining of the axolemma than of the surface membrane of Schwann cells. The criteria employed for the identification of UMAs were otherwise as given by Ochoa (1978). The micrographs were enlarged photographically to ×9000–10 500, measured and the data stored on the MOP Videoplan image analyser. For both MFs and UMAs the diameters of equivalent circles were calculated from measurements of fibre perimeter.

For determination of g ratios (axon diameter:fibre diameter; Bear and Schmitt, 1937) electron micrographs of up to 100 randomly selected fibres photographed at ×8000 were used. From the negatives, a myelin spacing factor was obtained by counting the number of myelin lamellae over a given width. This was repeated for 30 randomly selected fibres to provide a mean spacing factor. Myelin sheath thickness was then calculated from the total number of lamellae for each fibre multiplied by the spacing factor. This was repeated for each nerve. Axon diameter was determined from measurements of axonal circumference. Fibres sectioned through Schmidt-Lanterman incisures or in the paranodal region were excluded from this part of the study.

Using the method of Spencer and Thomas (1970), single fibres were isolated by teasing in Araldite without accelerator and mounted onto glass slides. As many fibres as possible (up to 100) were teased and categorized according to their morphological appearances. Under a Leitz optical microscope connected to the MOP Videoplan image analyser, mean fibre diameter (mean of three estimates of the widest internode) and internodal length were measured using a ×40 and ×25 objective, respectively, and then plotted against each other as described by Fullerton *et al.* (1965).

RESULTS

Clinical observations

All 19 patients had evidence of a distal sensory neuropathy. They were divided into the following three categories: Group 1, those with a severe autonomic neuropathy (10 cases); Group 2, those with chronic painful neuropathy and an accompanying autonomic neuropathy (4 cases); Group 3, those with an acute or chronic painful sensory neuropathy (5 cases).

Group 1. Autonomic neuropathy (Table 1). The 10 patients in this group (4 male, 6 female) had a mean age of 32.2 (range 23–39) yrs. Eight of the 10 patients had severe symptomatic neuropathy and in 7 of the 10, iritis had been diagnosed by ophthalmologists and treated with topical steroids. Autonomic function tests were severely abnormal in all patients, especially considering their age (all under 40 yrs). Only in 2 patients (Cases 1, 2) were heart rate changes on standing, together with Valsalva ratios, borderline. The severity of the somatic neuropathy on clinical assessment was variable. The most severe were Cases 2 and 7 with impairment of all sensory modalities in a glove and stocking

TABLE 1. CLINICAL DATA FOR PATIENTS WITH AUTONOMIC AND SENSORY NEUROPATHY (GROUP 1)

			Duration of	*Autonomic symptoms*[a]					*Autonomic tests*[b]						*Plasma creatinine*	
Case	*Sex*	*Age (yrs)*	*diabetes (yrs)*	*DD*	*GS*	*PH*	*G*	*B*	*HRV*	*PRS*	*VR*	*BPS*	*Retinopathy*[c]	*Proteinuria*	*(nr 60–120 μmol/l)*	*HbA1% (nr <10)*
1	F	23	10	+	+	+	–	–	6.1	17	1.2	0	B	No	53	16.0
2	F	30	16	+	+	+	–	+	5.2	16	1.15	10	P	No	106	13.0
3	F	39	27	+	+	+	–	+	4.0	8	1.0	50	P	No	133	15.0
4	M	38	19	+	+	+	+	+	1.5	7	1.06	50	P	Yes	62	11.8
5	F	38	17	+	+	+	+	+	4.0	0	1.0	50	P	Yes	177	11.8
6	M	27	13	+	+	+	–	–	4.0	22	1.08	37	P	Yes	106	20.0
7	M	33	16	+	+	+	–	–	7.3	9	1.0	50	P	No	106	10.5
8	M	30	11	+	+	–	–	–	4.0	23	1.07	31	P	No	71	12.4
9	F	32	17	–	–	–	–	–	0	10	1.07	23	B	No	80	9.6
10	F	32	18	–	–	–	–	–	4.0	4	1.14	35	P	Yes	75	11.3

nr = normal range. [a] DD = diabetic diarrhoea; GS = gustatory sweating; PH = postural hypotension; G = gastroparesis; B = bladder paresis. [b] HRV = heart rate variation on deep breathing (beats/min) (nr = 15 or more); PRS = change in pulse rate on standing (beats/min) (nr = 12 or more), VR = Valsalva ratio (nr = 1.21 more), BPS = drop in systolic blood pressure on standing (mmHg) (nr = 10 or less) [c] N = none; B = background; P = proliferative.

distribution. Four others had definite but milder sensory impairment distally in the lower limbs. In 4 (Cases 1, 8, 9, 10) there was minimal or no detectable sensory deficit. All cases had absent ankle reflexes; 6 cases (1, 2, 3, 7, 8, 10) also had absent knee jerks. A previous history of distal lower limb pain or paraesthesiae was obtained in 3 cases (1–3) dating back 3, 10 and 12 yrs, respectively, but without recurrence. Case 5 had occasional shooting pains in her feet at the time of biopsy.

The impairment of autonomic function was based on heart rate and blood pressure tests as outlined above, and all 10 patients had severe involvement. In 2 cases (9, 10) there were no symptoms suggestive of autonomic dysfunction despite severe abnormalities of formal testing.

Radiographs of the feet were taken in 8 patients (Cases 1–8) to assess the presence of arterial calcification; this was positive in 5 (Cases 2–6). Neuropathic foot ulcers were documented in 4 cases (5, 7, 9, 10); Case 1 had impaired sensation around an area of trauma on the dorsum of one foot, suggesting the early onset of a neuropathic ulcer; 2 cases (3, 10) had Charcot ankle joints.

Group 2. Autonomic and chronic painful neuropathy (Table 2). There were 4 female patients (Cases 11, 12, 13) in this group with a mean age of 40 (range 23–53) yrs and a duration of painful symptoms that ranged from 18 months to 6 yrs. The symptoms included painful burning sensations with tingling paraesthesiae affecting the feet. The occurrence of fingertip paraesthesiae was an additional symptom in Case 13. A history of weight loss at the onset of the painful symptoms was obtained in Cases 11 and 13. Cooling the feet provided the most satisfactory relief. Contact hyperaesthesia and insomnia were prominent in Case 11. All 4 cases had evidence of a severe autonomic neuropathy on testing, although Case 11 had no autonomic symptoms. All 4 patients had impairment of all sensory modalities in the lower limbs with absent ankle jerks. In Case 13 there was also reduced pin prick and light touch sensation in the hands. A neuropathic foot ulcer was present in this patient and also in Case 14. Arterial calcification in the foot was documented on radiographs of all 4 cases.

TABLE 2 CLINICAL DATA FOR PATIENTS WITH CHRONIC PAINFUL AND AUTONOMIC NEUROPATHY (GROUP 2)

					Autonomic symptoms[a]					*Autonomic tests*[b]							
Case	*Sex*	*Age (yrs)*	*Duration of diabetes (yrs)*	*Duration of pain (yrs)*	*DD*	*GS*	*PH*	*G*	*B*	*HRV*	*PRS*	*VR*	*BPS*	*Retinopathy*[c]	*Proteinuria*	*Plasma creatinine (nr 60–120 µmol/l)*	*HbA1% (nr < 10)*
11	F	23	8	4	–	–	–	–	–	3.0	14	1.1	20	B	No	44	11.5
12	F	45	20	6	+	+	–	–	–	5.0	14	1.2	26	B	No	71	11.0
13	F	53	32	4	+	+	–	–	–	2.0	6	1.03	17	B	No	71	11.8
14	F	39	28	1.5	+	–	–	–	–	4.0	9	1.1	36	P	Yes	144	12.9

[a], [b], [c] as for Table 1.

Group 3. Chronic and acute painful neuropathy (Table 3). Five cases were included in this group (2 male, 3 female); their mean age was 37.2 (range 18–54) yrs. Two female patients (Cases 15, 16) had chronic painful neuropathies with a 3 and 15 yr history of symptoms, respectively. Sensory testing was normal but the ankle reflexes were lost. Arterial calcification was not detected and neither case had evidence of neuropathic

TABLE 3. CLINICAL DATA FOR PATIENTS WITH PAINFUL SENSORY NEUROPATHY (GROUP 3)

									Autonomic tests[b]			
Case	*Sex*	*Age (yrs)*	*Duration of diabetes (yrs)*	*Duration of pain (yrs)*	*Retinopathy*[c]	*Proteinuria*	*Plasma creatinine (nr 60–120 µmol/l)*	*HbA1 % (nr < 10)*	*HRV*	*PRS*	*VR*	*BPS*
Chronic painful neuropathy												
15	F	31	8	3	N	No	71	15.0	15	22	1.6	9
16	F	42	11	15	B	No	80	9.0	16	12	ne	0
Acute painful neuropathy												
17	F	18	11	2 mos	N	No	60	8.5	20	24	ne	0
18	M	54	1	2 mos	N	No	64	6.2	ne	ne	ne	0
19	M	41	1	3 mos	N	No	ne	ne	ne	ne	ne	0

[b] and [c] as for Table 1; ne = not examined.

complications. In both patients the pain was confined to the feet and described as burning and tingling with troublesome hyperaesthesia. Insomnia was a prominent complaint in Case 15.

Three had an acute painful neuropathy. In 2 (Cases 17, 18) the symptoms were precipitated by improvement of glycaemic control. In one, a brittle diabetic, onset of the symptoms followed the initiation of treatment by continuous subcutaneous insulin infusion. This case has previously been reported in detail (Llewelyn *et al.*, 1986). The other was a recently diagnosed diabetic unsuccessfully managed on oral hypoglycaemic agents whose symptoms followed 4–6 wks after the commencement of insulin treatment. He initially developed paraesthesiae in both feet that later affected his hands. He had also become impotent. Abnormalities on examination of this patient revealed only mild impairment for temperature and pin prick sensation over the feet, and ankle reflexes obtained only with reinforcement. The third patient (Case 19) presented with an acute painful neuropathy associated with severe weight loss and impotence over 3 months. The only abnormality on neurological examination was an unpleasant burning response to pin prick and light touch over the distal part of his legs.

Nerve conduction studies

The results for motor and sensory conduction are given in Tables 4 and 5, respectively. The most consistent changes were found in the legs with absent or small sural sensory action potentials (SAPs) and abnormal tibial motor nerve conduction. Only in Case 16 was the sural sensory study completely normal; the median SAP was reduced in amplitude but this may have been caused by asymptomatic entrapment of the nerve at the wrist. Sural nerve conduction velocity was borderline in Case 18, in whom action potential amplitude was normal.

Quantitative sensory testing (Table 6)

Thermal thresholds in the foot were abnormal in 16/18 cases, but only 4/17 had clearly abnormal thresholds in the hand (with a borderline abnormal result in a further 4 cases). Vibration thresholds were abnormal in 12/18 cases when tested on the foot, but only clearly abnormal in 4 cases (with 3 other borderline results) when tested on the hand.

There were 4 cases (1, 9, 15, 18) in whom quantitative sensory testing revealed only

TABLE 4. MOTOR NERVE CONDUCTION

	Median				*Tibial*			
Case	*dl*	*ap*	*cv*	*F*	*dl*	*ap*	*cv*	*F*
Group 1								
1	ne	7.5	48.0	29.0	ne	ne	32.0	ne
2	ne	6.5	ne	30.0	Absent			
3	5.6	ne	47.0	ne	Absent			
4	4 3	3.5	42.0	34.0	Absent			
5	ne				6.5	1.10	34.0	63
6	4.5	7.5	42.0	31 0	7.2	0.35	ne	75
7	4.5	3.0	39.0	41.0	ne	0 20	24 0	ne
8	4.4	1 8	52.0	32 0	6.5	1.00	ne	66
9	3.2	4.5	51.0	32 0	4.8	2 00	ne	49
10	3 4	5 5	48 0	29 0	Absent			
Group 2								
11	ne	3.0	50.0	ne	ne	5.50	36.0	59
12	ne	8.0	43.0	31.0	ne	ne	27 0	ne
13	ne				ne			
14	ne	12.0	47.0	31.0	ne	ne	25.0	ne
Group 3								
15	3.4	8.0	51.0	ne	ne	ne	35 0	ne
16	3.2	14.0	46.0	30.0	7.1	2.00	ne	35
17	3 8	15.0	49.0	24 0	6.9	0.50	28 0	82
18	ne				5.7	5.10	40 0	54
19	ne	ne	51.0	27 0	Absent			
Controls	<4.2	>5 0	>48.0	<31.0	<4.2	>2.00	>40 0	–

dl = distal latency (ms); ap = action potential amplitude (mV); cv = conduction velocity ($m \cdot s^{-1}$); F = F wave latency (ms); ne = not examined.

thermal threshold abnormalities in the foot. All these cases had either no sensory loss or only minimal sensory deficits on clinical examination. Of the other 5 cases with only minor clinical sensory abnormalities (8, 10, 11, 16, 17), 3 had raised thermal and vibration thresholds (8, 10, 11), and 2 of the cases with acute painful neuropathy (16, 17) had normal quantitative tests. There was no case where thermal threshold was normal with an abnormal vibration threshold.

In the hand, changes of thermal and vibration thresholds were more closely related to each other than in the foot, with both parameters more or less equally normal or abnormal.

Qualitative findings

Light and electron microscopic examination showed a moderate to severe loss of MFs in the biopsies from all 19 cases, with a variable number of regenerated myelinated fibres (RMF). Active fibre breakdown or demyelination was rarely seen. In over half the cases, degenerated fibres retained a 'rigid' circular basal lamina (King *et al.*, 1989) which was either empty or contained regenerating axons. Apart from an increase in endoneurial collagen, the connective tissues appeared normal; no inflammatory infiltrates were seen. There was evidence of thickening of the walls of endoneurial blood vessels with reduplication of the basal lamina, as compared with the control cases, but all lumina

TABLE 5 SENSORY NERVE CONDUCTION

Case	Median ap	Median cv	Radial ap	Radial cv	Sural ap	Sural cv
Group 1						
1	17.0	ne	17.0	48.0	Absent	
2	5.0	58.0	11.0	ne	Absent	
3	4.0	47.0	3.0	40.0	Absent	
4	ne		3.0	ne	Absent	
5	ne		ne		Absent	
6	18.0	49.0	15.0	46.0	Absent	
7	ne		Absent		Absent	
8	5.0	52.0	10.0	48.0	Absent	
9	10.0	64.0	17.0	53.0	1.0	ne
10	13.0	62.6	30.0	56.0	Absent	
Group 2						
11	16.0	55.0	21.0	39.0	6.0	37.0
12	8.0	51.0	10.0	37.0	Absent	
13	ne		ne		ne	
14	11.0	60.0	9.0	37.0	Absent	
Group 3						
15	12.0	55.0	12.0	53.0	Absent	
16	9.0	64.0	ne		21.0	48.0
17	8.2	46.0	ne		3.0	36.0
18	ne		ne		15.0	38.0
19	9.0	ne	15.0	ne	Absent	
Controls	>15.0	>48.0	>20.0	>48.0	>10.0	>40.0

ap = action potential amplitude (μV); cv = conduction velocity ($m \cdot s^{-1}$); ne = not examined.

were patent. No intraluminal fibrin or platelet 'plugs' were encountered and there was no indication of vasculitis. There was no evidence of nerve oedema.

Myelinated fibre morphometry (Table 7, fig. 1). The mean fascicular area examined for all diabetic cases was 0.24 mm^2 and that for the controls was 0.32 mm^2. There was in general a uniform loss of large and small diameter fibres which increased with increasing severity of neuropathy (figs 1, 2).

Group 1 patients (Cases 1–10) had the most severe loss of myelinated fibres (range 737–3135 MF/mm^2) as compared with controls (range 7172–11571 MF/mm^2). Of those remaining, a high percentage (mean 84%; range 49–100%) were less than 7 μm in diameter; a large proportion of these were RMFs in regenerative clusters (mean no. of clusters/mm^2 255; range 35–510). A regenerative cluster was defined as 2 or more closely apposed myelinated fibres. These were often of small diameter with relatively thin myelin sheaths and surrounded by proliferated Schwann cells, sometimes enclosed within a persisting basal lamina. The percentage of RMFs of the total MF count ranged from 3.4 to 70.7 with a mean of 33.6. There was an overall trend for the number of RMFs to be greatest with falling MF density, but when this went below 1750 MF/mm^2 the number of RMFs became sharply reduced (Table 7, fig. 3). The mean ratio of small:large fibres (< 7 μm: > 7 μm) for all patients together was 5:1. If the RMFs (i.e., in clusters) are excluded, the ratio is less at 3.1:1, but still significantly greater

TABLE 6 THERMAL AND VIBRATION THRESHOLDS

Case	*Thermal threshold (°C)**		*Vibration threshold (V)*	
	Hand	*Foot*	*Hand*	*Foot*
Group 1				
1	2.1	>30	4.3	8.6
2	3.5	>30	4.0	>50
3	6.0	>30	6.0	>40
4	11.0	>30	8.7	38.0
5	>16	>30	8.0	>40
6	5.3	>30	6.0	36.0
7	>30	>30	36.0	>40
8	3.0	>30	6.0	11.0
9	4.5	>30	3.0	4.0
10	ne	>30	ne	14.0
Group 2				
11	2.8	25	4.0	11.0
12	2.8	18	5.0	17.6
13	5.8	>30	8.6	25.0
14	6.3	20	4.0	22.0
Group 3				
15	3.0	17	3.0	4.0
16	3.3	6	5.0	5.0
17	2.0	5	3.0	4.0
18	1.9	20	3.0	7.0
19	ne		ne	
Controls	<4.5	<9.5	<6.0	<10.6

* Warm thermal thresholds; ne = not examined.

than in controls where the mean ratio was 1.7:1. Isolated regenerated fibres would still be included.

In Group 2 (Cases 11−14) with severe autonomic neuropathy and chronic painful symptoms, there was again a severe loss of MFs (range 1073−3242 MF/mm^2) with marked regenerative activity (mean no. of clusters/mm^2 172; range 106−252), with RMFs accounting for a mean of 21.6% of the total MFs (range 12.0−30.8%). As in Group 1 there was loss of fibres of all sizes and of those remaining the majority were less than 7 μm in diameter. The mean ratio < 7 μm: > 7 μm was higher than controls at 3.0:1; excluding RMFs, the ratio was 2.1:1.

The patients with chronic painful (Cases 15, 16) and those with acute painful neuropathy (Cases 17−19) of Group 3 had a less severe depletion, again affecting all sizes of MFs (range 2684−6920/mm^2), as compared with the cases from Groups 1 and 2. There was considerable regenerative activity (mean no. clusters/mm^2 62, range 21−83) but much less than was found in Groups 1 and 2. The ratio of fibres < 7 μm: > 7 μm was similar to that of controls at 1.8:1 (1.7:1 after excluding RMFs).

There was a significant correlation between vibration threshold in the foot and the total MF count for all cases combined ($P < 0.001$) (fig. 4). There was an equally significant relationship between vibration threshold (foot) and the density of large MFs (> 7 μm diameter). The severity of the autonomic neuropathy taking heart rate variation

TABLE 7. MYELINATED FIBRE DATA

					Differential MF density					
			RMF		*< 7 μm*			*> 7 μm*		
Case	*Density (per mm²)*	*Clusters (per mm²)*	*(per mm²)*	*(%)*	*(per mm²)*	*(%)*	*(*%)*	*(per mm²)*	*(%)*	*(*%)*
Group 1										
1	3135	244	706	(22.6)	2165	(69)	(60)	970	(31)	(40)
2	1559	422	1102	(70.7)	1497	(96)	(86)	62	(4)	(14)
3	2786	41	96	(3.4)	2786	(100)	(100)	–	–	–
4	2700	313	790	(29.3)	2665	(99)	(98)	35	(1)	(2)
5	737	35	70	(9.5)	632	(86)	(85)	105	(14)	(15)
6	888	99	237	(26.7)	434	(49)	(30)	454	(51)	(70)
7	954	255	580	(60.8)	931	(98)	(97)	23	(2)	(3)
8	2277	510	1383	(60.7)	1995	(88)	(69)	282	(12)	(31)
9	2901	294	777	(26 8)	2573	(89)	(82)	328	(11)	(18)
10	3049	339	786	(25.8)	1851	(61)	(47)	1198	(39)	(53)
Mean	2099	255	653	(33.6)	1753	(84)	(74)	346	(16)	(26)
Group 2										
11	3242	178	508	(15.7)	1579	(49)	(39)	1663	(51)	(61)
12	2226	252	624	(28.0)	1382	(62)	(47)	844	(38)	(53)
13	1941	106	232	(12.0)	1770	(91)	(90)	171	(9)	(13)
14	1073	152	330	(30.8)	1037	(97)	(95)	36	(3)	(5)
Mean	2121	172	424	(21.6)	1442	(75)	(68)	679	(25)	(33)
Group 3										
15	2684	83	192	(7.2)	1508	(56)	(53)	1176	(44)	(47)
16	6920	74	194	(2.8)	5225	(76)	(75)	1694	(24)	(25)
17	4566	74	207	(4.5)	2684	(59)	(57)	1882	(41)	(43)
18	5358	59	117	(2.2)	3134	(59)	(56)	2224	(41)	(44)
19	6014	21	42	(0 7)	3933	(65)	(65)	2081	(35)	(35)
Mean	5108	62	150	(3.5)	3297	(63)	(61)	1811	(37)	(39)
Controls										
C1	9084	–	–		5170	(57)		3914	(43)	
C2	10096	–	–		6482	(64)		3614	(36)	
C3	7172	–	–		4798	(67)		2374	(33)	
C4	11571	–	–		7324	(63)		4247	(37)	
C5	10404	–	–		6888	(66)		3533	(34)	
Mean	9665	–	–		6132	(63)		3533	(37)	

MF = myelinated fibres; RMF = regenerating myelinated fibres, *% = percentage of MFs when RMFs are subtracted.

(HRV) as one index is closely related to depletion in MF density (fig. 5). Only 1 patient (Case 15, Group 3) had an MF density less than 4000/mm^2 and HRV within the normal range.

Unmyelinated axon morphometry (Table 8, fig. 6). The mean area examined for all cases was 0.023 mm^2. For the controls this was 0.021 mm^2.

The size-frequency distribution in our control nerves was unimodal with a median diameter of between 1 and 1.5 μm. Values for the normal density of UMAs reported in the literature have varied greatly. In our control group it ranged from 28363 to 33270/mm^2.

In Group 1, UMA density in general was markedly reduced, but there were some exceptions, specifically Cases 2, 5 and 7 where the UMA counts were greater than those of our normal controls. This increase is probably explained by the presence of regenerating

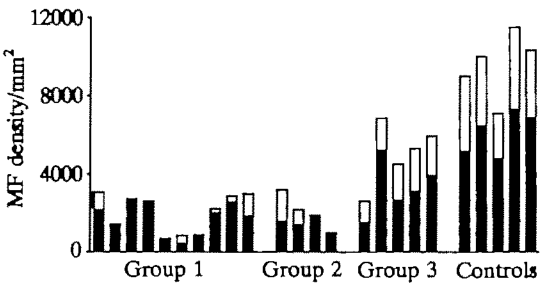

FIG 1 Myelinated nerve fibre (MF) density (per mm^2 fascicular area) in sural nerve from individual patients from Groups 1–3 and in control subjects. The filled portions of the columns indicate fibres < 7 μm and the open portions those > 7 μm in diameter.

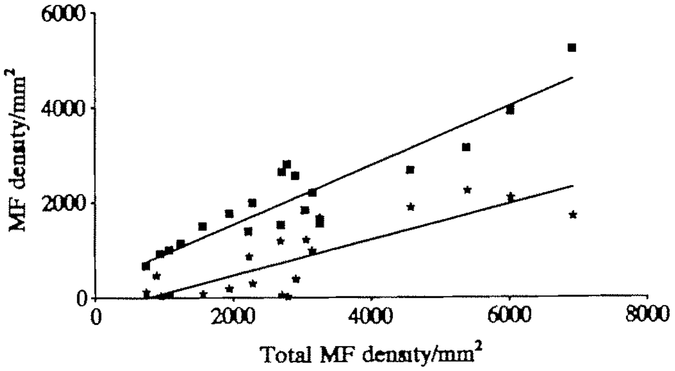

FIG. 2. Relationship between myelinated fibre (MF) density for fibres less (squares) and greater (asterisks) than 7 μm in diameter with calculated regression lines. For those with a diameter < 7 μm, $r = 0.93$, $P < 0.0001$; for those with a diameter > 7 μm, $r = 0.83$, $P < 0\ 0001$. Each pair of vertically related points represents one diabetic patient.

axons. There was a significant shift to the left in the histograms, towards UMAs with smaller diameters in all cases. A mean of 85.4% of axons had diameters less than 1 μm as compared with a mean of 33.6% in our controls. This shift towards smaller diameter UMAs is reflected in the mean of median diameter of 0.60 μm for the whole group as compared with the control value of 1.22 μm (fig. 7). This may represent either a loss of larger diameter unmyelinated axons, the presence of regenerating unmyelinated axons sprouts, or a mixture of both.

Because of technical difficulties with tissue sectioning for electron microscopy, no results were available for Case 11. Two cases (12, 13) of Group 2 had similarly reduced total UMA densities but again there was a shift towards smaller diameters. In Case 14, UMA density was raised, with a small median diameter. A mean of 83.5% of axons had a diameter of less than 1 μm. The mean of the median diameters was 0.73 μm. In Group 3, the shift of the UMA histograms to the left was not as marked as in the other two groups, with a mean of 45.8% UMAs less than 1 μm in diameter (control value was 33.6%) and a mean of median axon diameters at 1.04 μm. Cases with foot thermal thresholds above 20° C had a significantly lower median UMA diameter compared with those whose thermal thresholds were either normal ($< 10°$ C) or between 10 and 20° C (fig. 8, Table 9). When the total UMA count is plotted against total MF

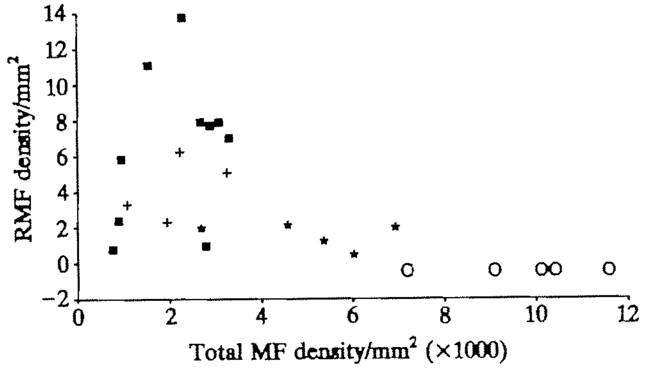

FIG. 3. Relationship between density of regenerated myelinated nerve fibres (RMF) per mm^2 fascicular area and total myelinated nerve fibre density for patients from Groups 1 (closed squares), 2 (crosses), 3 (asterisks) and controls (open circles).

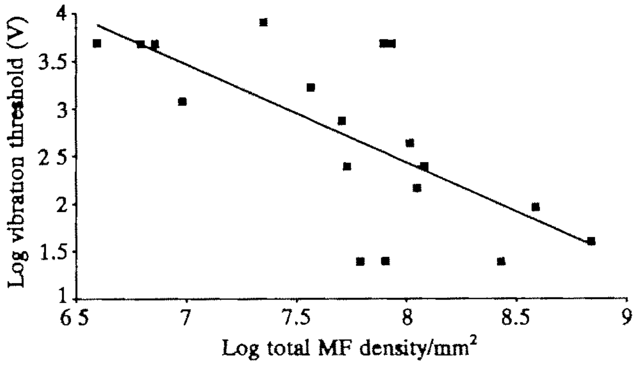

FIG. 4 Natural logarithmic relationship between sensory threshold for vibration at the hallux and myelinated nerve fibre (MF) density for the diabetic patients, with calculated regression line ($r = -0.70$, $P < 0.001$)

count both decrease uniformly until the MF count goes below 3000/mm^2, after which the total UMA counts become extremely variable (fig. 9). There is a significant correlation ($r = 0.72$, $P < 0.001$) between median UMA diameter and the total MF count with smaller UMA diameters at lower MF counts (fig. 10).

In the majority of cases from Groups 1 and 2, there was evidence of UMA loss. Stacks of flattened Schwann cell processes unassociated with axons (Ochoa, 1978) were numerous with little or no regeneration. Collagen pockets were also numerous (range 502−12367/mm^2). In Group 3, there was only minimal unmyelinated axon degeneration/regeneration, but again collagen pockets were frequent (range 994−7778/mm^2).

g ratios (Table 10). The scattergrams of g ratio against axon diameter highlight changes in sheath thickness in relation to axon diameter. The normal adult sural nerve has two distinct populations (Friede and Beuche, 1985), one consisting of thinly myelinated small

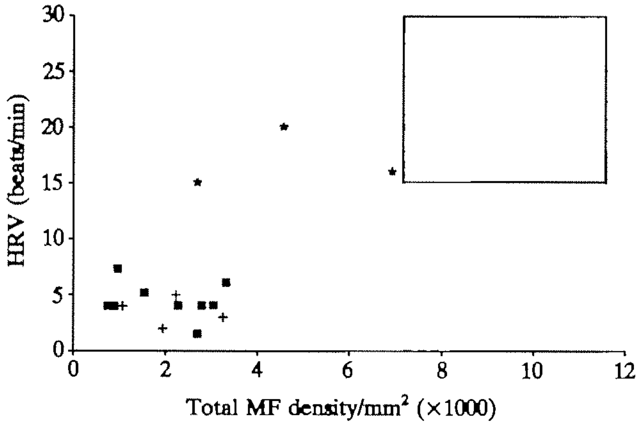

FIG. 5. Relationship between heart rate variation (HRV) and myelinated nerve fibre (MF) density for the diabetic patients. Groups 1, 2 and 3 cases are indicated as for fig 3 The boxed area indicates the range for control subjects Patients from Groups 1 and 2 have low HRV with reduced total MF density

diameter fibres (higher g ratios), and a second group made up of larger diameter fibres, the larger of which have thinner myelin sheaths (lower g ratios; fig. 11A). Because of the severity of MF loss, a satisfactory g ratio analysis was possible only in 11 cases. For those studied, there was a generally increased scatter of g ratio values, especially for the smaller diameter axons (fig. 11B). The percentage of fibres with a g ratio greater than 0.8 was increased compared with controls and the majority of these had axon diameters of less than 5 μm. There was no obvious trend between the three clinical groups. In cases where a severe depletion of larger MFs had occurred, only one cloud was seen in the scattergrams.

Teased fibre studies (Table 11). Teasing of individual fibres was possible in 13 cases. In the 5 other cases, fibres could not be isolated because of an excess of endoneurial collagen and the fact that the surviving fibres were mainly of very small diameter. The results were compared with the control values of Jacobs and Love (1985). There was an excess of abnormal fibres in all but 1 patient (Case 9) of those examined. Both segmental demyelination/remyelination and axonal degeneration/regeneration were encountered. No specific features could be identified within the clinical categories. Occasional fibres undergoing acute axonal degeneration with breakdown of the myelin sheath into ovoids were seen but they were not more frequent in the patients with painful neuropathy. Paranodal abnormalities, suggesting restricted paranodal demyelination and remyelination, were frequent.

The relationship between internodal length and fibre diameter is approximately linear in normal adults aged 60 yrs or less (Lascelles and Thomas, 1966; Jacobs and Love, 1985). The scatter of internodal lengths on individual fibres (fig. 12A) was increased in 8 out of the 12 cases examined. Many fibres possessed both long and short internodes, suggesting remyelination after segmental demyelination. Some of the larger fibres had uniformly shorter internodes resulting in a flattening of the graph, as in fig. 12B. These probably represent regeneration after axonal degeneration (Fullerton *et al.*, 1965).

TABLE 8 UNMYELINATED AXON DATA

Case	*Density* (*per mm²*)	*UMA* < 1.0 *μm*		*Median UMA diameter* (*μm*)
		(*per mm²*)	(*%*)	
Group 1				
1	15304	14271	(93)	0.55
2	37020	24940	(92)	0.55
3	23130	16607	(72)	0.71
4	8264	7140	(86)	0.62
5	44578	41413	(93)	0.51
6	13590	11620	(86)	0.61
7	43407	38198	(88)	0.60
8	23016	21520	(94)	0.48
9	3482	2946	(85)	0.50
10	12425	8076	(65)	0.85
Mean	22421	18673	(85.4)	0.60
Group 2				
11	–	–		–
12	22857	18720	(82)	0.68
13	22170	18889	(85)	0.60
14	45272	25760	(60)	0.91
Mean	30100	21123	(75.7)	0.73
Group 3				
15	8011	3717	(46)	1.04
16	27581	7750	(28)	1.26
17	17700	12195	(69)	0.77
18	14457	5450	(38)	1.10
19	19846	9586	(48)	0.80
Mean	17519	7740	(45.8)	0.99
Controls				
C1	29464	11196	(38)	1.14
C2	33270	16702	(50)	1.00
C3	28363	8622	(30)	1.19
C4	30546	9469	(31)	1.28
C5	28716	4882	(19)	1.48
Mean	30072	10174	(33.6)	1.22

UMA = unmyelinated axons.

DISCUSSION

This large study of sural nerve morphometry on patients with diabetic autonomic and painful neuropathies has demonstrated extensive loss of large and small myelinated fibres to be most pronounced in those with severe autonomic involvement. The distribution of fibre changes correlates well with various clinical assessments although some patients without detectable sensory loss still had moderately extensive fibre loss, for example, Case 9. However, quantitative sensory testing in these patients was highly abnormal. Regenerative activity of MFs showed no relationship with the presence either of acute or chronic painful symptoms.

In our clinical assessment, apart from the neurological examination, we undertook nerve conduction studies, quantitative vibration and thermal sensory testing, and

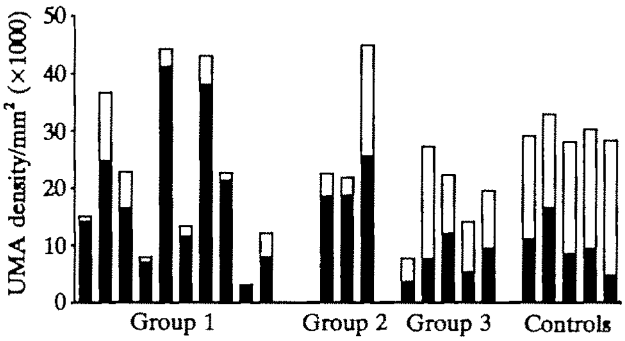

FIG. 6. Unmyelinated axon (UMA) density (per mm² fascicular area) in sural nerve from individual patients from Groups 1–3 and in control subjects. The filled portions of the columns indicate fibres < 1 μm and the open portions those > 1 μm in diameter.

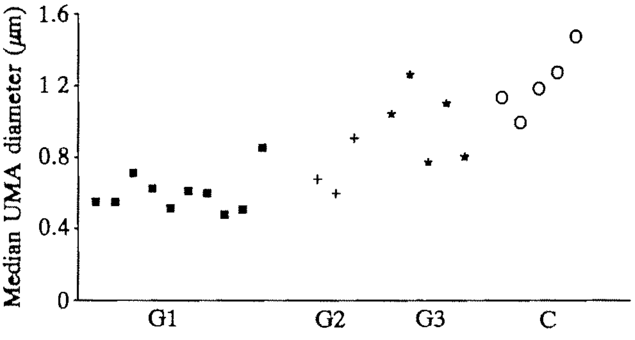

FIG. 7. Median diameter for unmyelinated axons (UMA) for patients from Group 1 (G1) (closed squares), 2 (G2) (crosses), 3 (G3) (asterisks) and controls (C) (open circles).

quantitative tests of autonomic function. Conventional nerve conduction studies reflect large MF function and may therefore be normal in cases with selective small fibre involvement. Vibration sense testing also assesses large MF function. We did not use separate measurements of warm and cold perception although, as there are differences in the innervation, distribution and density of warm and cold receptors (Light and Perl, 1984), either parameter could be differentially affected in diabetic subjects. Cold receptors are innervated by small (A delta) myelinated nerve fibres and warm receptors by unmyelinated C fibres. Cold perception threshold was found by Claus *et al.* (1987) to be more sensitive than warm perception threshold in diabetic neuropathy. The same conclusion was reached by Ziegler *et al.* (1988) in their study on newly diagnosed young insulin-dependent diabetics without clinical evidence of neuropathy.

In much older diabetic patients, unselected other than for the presence of neuropathy, Dyck *et al.* (1985, 1987) found that lower limb vibration sense was more frequently abnormal than the thermal cooling threshold, whereas Guy *et al.* (1985) found that thermal perception was more often abnormal than vibration perception in diabetic neuropathy.

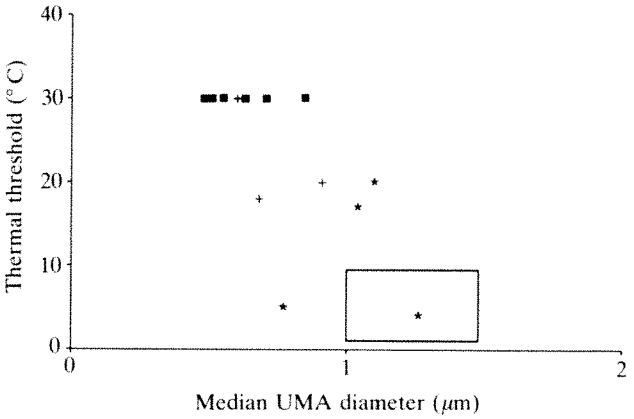

FIG. 8. Relationship between foot thermal threshold and median unmyelinated axon (UMA) diameter for the diabetic patients. Groups 1, 2 and 3 are indicated as for fig. 3. The boxed area indicates the control range. Those patients with thermal thresholds > 20° C had significantly lower mean of median UMA diameters than those with thermal threshold values between 10 and 20° C and less than 10° C (*see* Table 11).

TABLE 9. FOOT THERMAL THRESHOLDS AND MEDIAN UMA DIAMETERS

No. of patients	*Foot thermal thresholds (° C)*	*Mean of median UMA diameters (μm) (±SD)*
11	> 20	0.58 ± 0.07
4	10–20	0.93 ± 0.19*
2	< 10	1.02 ± 0.35*

* Significant difference at the $P < 0.05$ level (using the Scheffe procedure) compared with patients with thermal thresholds > 20° C.

In our series of selected patients foot thermal thresholds were more frequently abnormal than vibration thresholds. This probably reflects the deliberate inclusion of patients with prominent autonomic symptoms and painful sensory neuropathy, in both of which involvement of small fibres would be expected, as will be discussed later. In the hand, thermal and vibration parameters were less severely abnormal, but more closely related to each other, than in the foot.

Our results showed a correlation between vibration threshold and the total number of MFs (fig. 4). The same was true for large (> 7 μm) but not for the small MFs. There was also a correlation between thermal threshold and the median UMA diameter (fig. 8), but not with the total UMA or MF count.

Two of our cases (16, 17) had temperature and vibration thresholds that were within normal limits, 1 of whom (Case 16) also had a normal sural nerve electrophysiological study. In this latter case, there was a mild reduction both in the MF and UMA counts and increased numbers of RMFs. It is possible that the neuropathy could have been confirmed electrophysiologically if a more distal sensory nerve had been examined, such as the medial plantar nerve, as this has been documented to be frequently absent in diabetic subjects even when sensory threshold tests are normal (Levy *et al.*, 1987).

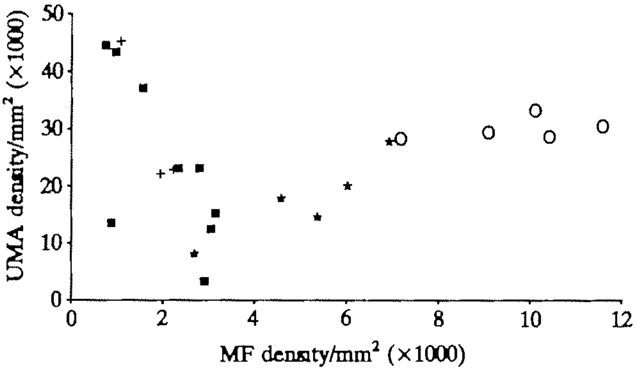

FIG 9 Relationship between unmyelinated axon (UMA) and myelinated fibre (MF) densities for Group 1 (closed squares), Group 2 (crosses), Group 3 (asterisks) and controls (open circles).

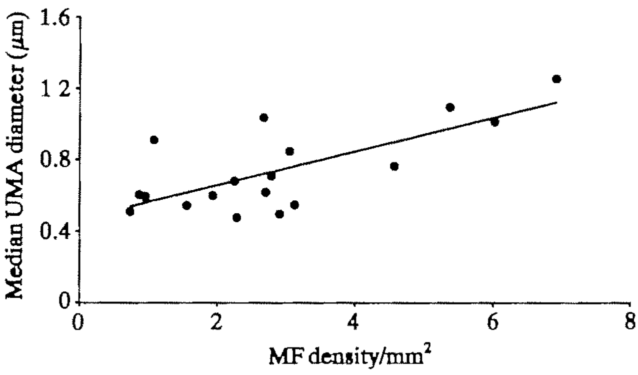

FIG. 10. Relationship between median unmyelinated axon (UMA) diameter and myelinated fibre (MF) densities with calculated regression line ($r = 0.72$, $P < 0.001$)

Neuropathic foot ulceration in diabetic neuropathy

Four cases (5, 7, 9, 10) with established neuropathic ulcers were included within our Group 1 patients. Case 1 had an 'early' neuropathic foot ulcer; 2 cases (13, 14) from Group 2 also had neuropathic foot ulcers.

Painless plantar foot ulcers in diabetic patients are usually associated with clinically severe distal sensory loss, although sensory abnormality is unlikely to be the only factor involved (Delbridge *et al.*, 1985). This is clearly illustrated in 2 of our cases, where clinical sensory testing was either normal or only minimally abnormal. At the time when the foot ulcer became apparent, 1 patient had constant painful symptoms in the lower limbs, with another (Case 5) having intermittent 'shooting pains'. The others were asymptomatic. The importance of an associated severe autonomic neuropathy has been documented (Deanfield *et al.*, 1980; Edmonds *et al.*, 1981), and this was the case in

TABLE 10 *g* RATIO DATA

Case	*n*	$g > 0.8$ *n*	$g > 0.8$ %
Group 1			
1	75	11	14.7
2	68	4	5.9
6	24	5	20.8
8	76	3	4.0
10	100	24	24.0
Group 2			
12	100	14	14.0
14	76	16	21.1
Group 3			
15	77	20	26.0
16	75	7	9.3
17	100	23	23.0
18	93	8	8.6
19	103	6	5.8
Controls			
C1	100	6	6.0
C2	100	5	5.0
C4	100	5	5.0
C5	90	5	5.6

n = no. of myelinated fibres assessed

all 7 of our patients. Sweat volume production, as a test of lower limb autonomic function, is highly abnormal in diabetics with neuropathic ulcers (Ahmed and Le Quesne, 1986). Quantitative sensory testing showed highly abnormal thermal thresholds in our 7 cases, but vibration threshold was normal in 2 cases (1, 9) and just above the upper limit of the normal range in another (10).

The study by Guy *et al.* (1985) did not separate the sensory threshold results of the patients with neuropathic ulcers from the other patients with diabetic neuropathy, but the conclusions are similar to ours in that thermal threshold is more consistently abnormal than vibration threshold in these patients. More recently, Ali *et al.* (1989) also reported more abnormal results with thermal thresholds than with vibration thresholds in those with neuropathic ulcers. They also found that thresholds for warming were more sensitive than those for cooling and that sensory threshold abnormalities were bilateral even when the ulceration was confined to one side.

There were no specific sural nerve morphological abnormalities that distinguished those with neuropathic ulcers from the other cases with severe autonomic neuropathy. All had a variably severe loss of MFs with an excess of RMFs, and a high percentage of small MFs (< 7 μm). Total UMA counts were also variable, with 3 cases (5, 7, 14) having densities above our normal range; the others had severe depletion of UMAs. It may be that at a site closer to the ulcer than the retromalleolar sural nerve, sensory nerve morphometry would have been more uniformly abnormal.

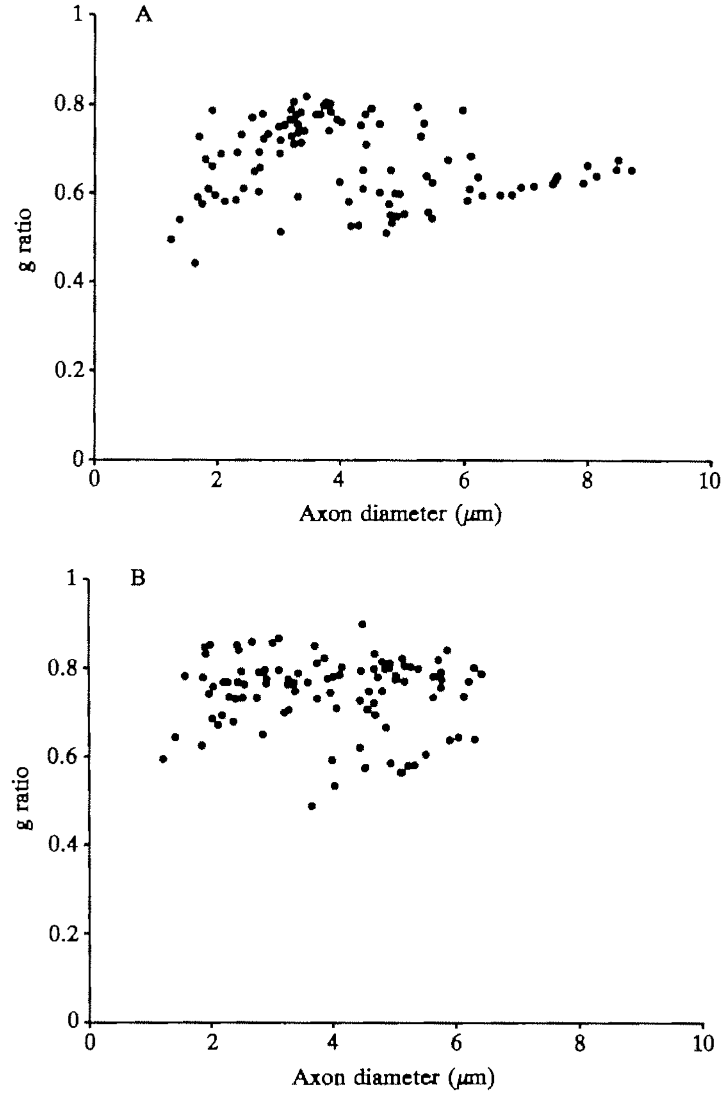

FIG. 11. A, relationship between g ratio and axon diameter for a control subject aged 38 yrs, showing one population (*upper left*) with smaller axon diameter and relatively thinner myelin sheaths, and a second population (*lower right*) with a larger axon diameter, the larger of which possess relatively thinner myelin sheaths B, relationship between g ratio and axon diameter for a patient aged 32 yrs from Group 1 (*Case 10*). The proportion of fibres with a g ratio > 0.8 is increased. There are no fibres with an axon diameter > 7 μm

The nature of the nerve fibre pathology in diabetic polyneuropathy

Distally accentuated axonal degeneration is the salient change in diabetic polyneuropathy. This affects both myelinated and unmyelinated axons (Martin, 1953; Greenbaum *et al.*, 1964; Thomas and Lascelles, 1966; Olsson *et al.*, 1968; Behse *et al.*, 1977). Sugimura and Dyck (1982) and Dyck *et al.* (1986*a*) found evidence from autopsy

TABLE 11. TEASED FIBRE ABNORMALITIES

Case	*Normal (%)*	*Segmental demyelination/ remyelination (%)*	*Acute axonal degeneration (%)*	*Axonal regeneration (%)*	*Paranodal changes (%)*
Group 1					
6	50	20	6	13	11
7	80	5	3	11	1
8	81	7	1	5	6
9	100	–	–	–	–
10	87	1	–	8	4
Group 2					
11	71	10	9	5	5
12	86	2	–	3	9
13	53	6	6	29	6
Group 3					
15	70	6	1	13	10
16	72	19	–	–	9
17	85	4	–	11	–
18	81	–	–	19	–
19	76	2	2	8	14

studies that proximal multifocal lesions, considered to have arisen on a vascular basis, could summate to produce a diffuse distally accentuated fibre loss. The patients studied, however, were elderly, and superimposed vascular lesions could have given rise to multifocal lesions on top of a diffuse neuropathy. Indeed, there was evidence both of multifocal and diffuse fibre loss, the diffuse loss being greater in posterior than in anterior spinal roots. In a series of nerve biopsies from patients with diabetic neuropathy, again from older patients, Dyck *et al.* (1986*b*) found evidence of multifocal fibre loss, taken as being suggestive of a vascular causation. Yet Llewelyn *et al.* (1988) found a similar patchy loss of fibres in sural nerve biopsies from a younger group of cases with diabetic neuropathy, and showed an identical pattern of fibre loss in a group of patients with hereditary motor and sensory neuropathy matched for age and severity of fibre loss. This pattern of fibre loss therefore cannot be taken as unequivocal evidence of ischaemic damage.

The alternative view to multifocal pathology related to nerve ischaemia is a distal axonopathy secondary to metabolic effects on the axons or their parent cell bodies. Teased fibre studies by Said *et al.* (1983) in cases of diabetic 'small fibre' neuropathy produced evidence of a 'dying-back' process. It is not yet certain whether diabetic neuropathy could represent a central-peripheral distal axonopathy (Spencer and Schaumburg, 1976). Dolman (1963) showed that loss of fibres also occurs in the posterior columns of the spinal cord but it is not known whether this has a rostral accentuation.

The axonal loss is accompanied by segmental and paranodal demyelination (Thomas and Lascelles, 1966; Behse *et al.*, 1977). Dyck *et al.* (1980) found that, in untreated diabetics without symptoms of neuropathy, an increased incidence of segmental demyelination and remyelination was observed. Untreated diabetics with symptomatic neuropathy showed both demyelination and remyelination and axonal degeneration. Finally, in treated diabetics, often with longstanding disease, axonal degeneration

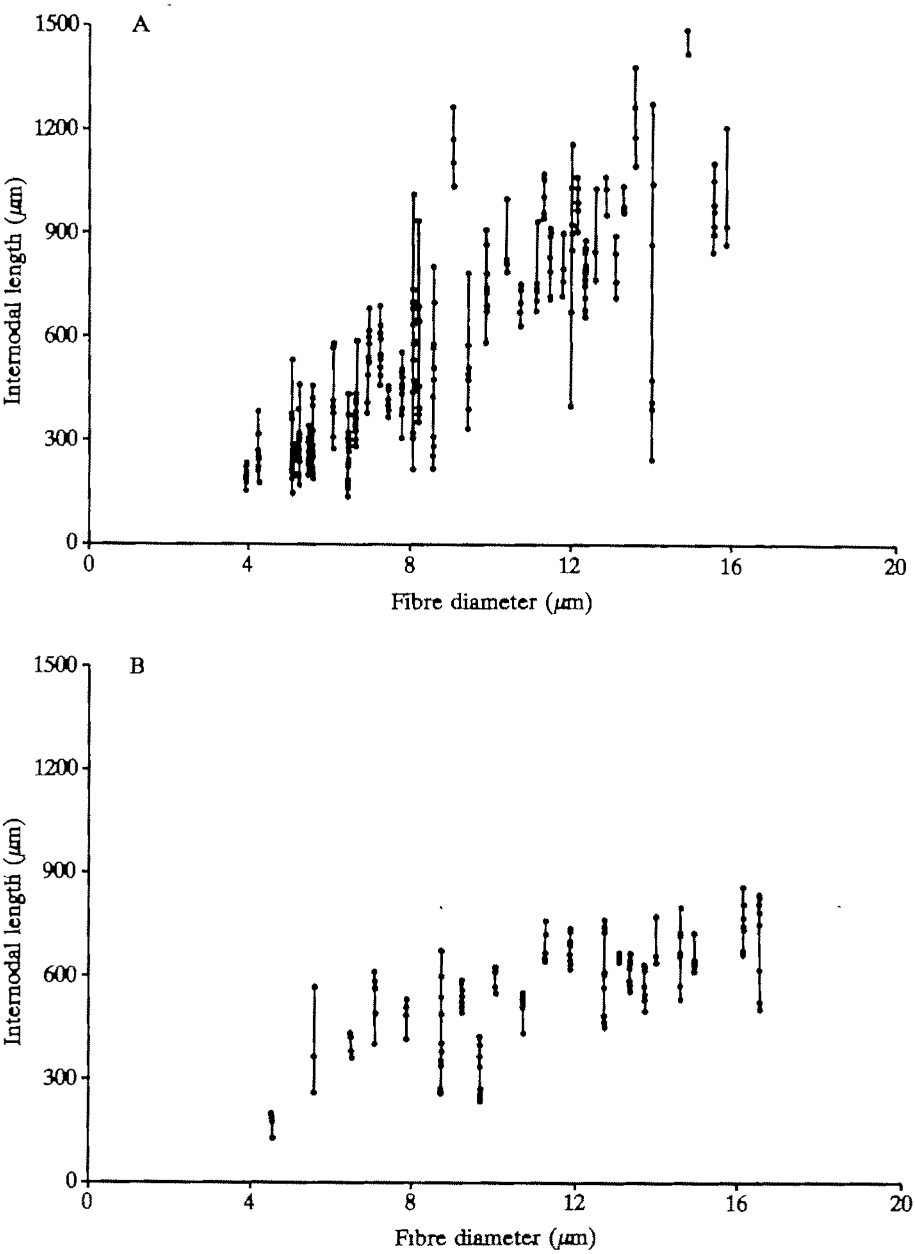

FIG. 12. Distribution of internodal lengths on individual fibres, A, for a patient aged 42 yrs from Group 3 showing considerably increased variability and B, for a patient aged 31 yrs from Group 3 showing a reduced number of longer internodes. Each vertical line represents a single fibre.

predominated. Our biopsies showed a combination of axonal degeneration and regeneration and demyelination and remyelination. It is not yet clear whether the demyelination is of the primary or secondary type. Behse *et al.* (1977) and Yagihashi and Matsunaga (1979) found little correlation between axonal degeneration and the extent of demyelination. On the other hand, secondary demyelination has been observed in teased fibres proximal to distal axonal degeneration by Said *et al.* (1983). Secondary demyelination may be the consequence of axonal atrophy. Sugimura and Dyck (1981) found no evidence that axonal atrophy precedes segmental demyelination in diabetic neuropathy. The observations on the g ratios obtained in the present study again give no indication that the occurrence of axonal atrophy is an important component of diabetic neuropathy. Myelin proteins are abnormally glycosylated in diabetes. Vlassara *et al.* (1985) have found that macrophages recognize and interact with glycosylated myelin products and have suggested that this may contribute to segmental demyelination.

Axonal regeneration was a prominent feature of our biopsies, as has been observed by others. There was no clear relationship between the number of RMFs and the total number of MFs and the trend in our cases was for the number of RMFs to be largest with the greatest loss of MFs (fig. 3) until the MF density fell to 1750/mm^2, below which the number of RMFs tended to fall. This differs from observations in a recent report by Dyck *et al.* (1988) where a clear negative correlation was found, in that a lower number of RMFs was found with greater fibre loss. The main distinguishing features between our cases and those of Dyck *et al.* (1988) are younger age (mean 36.6 vs median 50.0 yrs) and the presence of severe autonomic neuropathy in 13/18 of our cases. Further studies are required to elucidate what factors govern the rate and extent of myelinated fibre regeneration in diabetic neuropathy.

Because of the wide range of values for total unmyelinated axon counts in control subjects (19 000 – 65 000/mm^2 (Ochoa, 1978); 30 000 – 40 000/mm^2 (Jacobs and Love, 1985)), and the fact that at the present time it is not possible to distinguish histologically between sensory and autonomic axons in the sural nerve or to distinguish with certainty regenerating from nonregenerating unmyelinated axons, interpretation of total unmyelinated axon counts is difficult. Another problem encountered in advanced chronic neuropathy is in distinguishing those unmyelinated axons that will at a later stage become myelinated from those that will remain unmyelinated.

The regenerating axons, when relatively mature, were often present within the persisting basal lamina that had surrounded the intact nerve fibre. This frequently had a circular configuration in transverse section instead of the usual collapsed crenated contour that develops after axonal degeneration. These findings suggest abnormal rigidity and persistence of the Schwann cell basal lamina in diabetes (King *et al.*, 1989). Such persistent basal laminal tubes often contain densely packed collagen fibrils. The progressive collagenization of nerve trunks in diabetic neuropathy could contribute to failure of effective regeneration (*see* King *et al.*, 1989).

Pain in diabetic neuropathy

Painful symptoms, consisting of intermittent or protracted aching, stabbing sensations and painful burning paraesthesiae, are frequently encountered in diabetic neuropathy. These are typically worse at night. Troublesome contact hyperaesthesia (allodynia) of the skin may also be present. Treatment is difficult, with no consistent relief by any medication except potent analgesics which are generally not indicated. Cooling of the feet often provides temporary relief, but cold injury has been reported following its excessive use (Allen and MacDonald, 1987). Our series included patients both with acute and chronic pain.

In diabetic patients with pain, the severity of somatic neuropathy is often clinically mild. In the present series the pathological changes were generally less severe in those with pain. Those with an associated severe autonomic neuropathy had a more profound fibre loss. The cases included in Group 2 share a number of clinical features with those described by Brown *et al.* (1976) and some of those reported by Said *et al.* (1983) with painful neuropathy, autonomic involvement, depressed or absent ankle jerks and relative preservation of large fibre modalities on clinical examination. Quantitative examination of thermal and vibration threshold did, however, show a clear abnormality in all our cases. There was little uniformity in the morphometric parameters within this group, but as the severity of the MF depletion increased, so did the percentage of small (< 7 μm diameter) fibres that remained (from 49% to 97%). Many of these were probably regenerating. The main feature for the UMAs was the leftward shift of the median diameter in favour of those of smaller diameter, as was found by Brown *et al.* (1976). The same conclusion emerges from the patients in Group 1 with severe autonomic neuropathy but little sensory loss (Cases 1, 8–10). The reduced median diameter of unmyelinated axons could represent the presence of small regenerating axons, but this does not correlate with the occurrence of pain. Median UMA diameter was greatest in Group 3 cases (Table 8).

Our findings therefore suggest that between patients with clinically a 'small fibre' neuropathy there is considerable variation in MF and UMA distributions. This is in keeping with the conclusion of Dyck *et al.* (1986*b*) following a morphometric study of sural nerve biopsies from 32 patients with diabetic neuropathy when they failed to identify a selective loss of large or small myelinated fibres. It was suggested that cases of 'small fibre' and 'large fibre' diabetic neuropathy are the result of the artificial separation of cases from either end of a continuous distribution. However, the assessment of the severity of loss of particular fibre groups by the inspection of size-frequency histograms is handicapped by the presence of large numbers of regenerated and remyelinated fibres, as indicated by the results of the teased fibre studies. The removal of fibres in regenerative clusters does not eliminate this difficulty. Precise correlations between clinical features and patterns of fibre loss must therefore be approached with caution in the presence of prominent regenerative activity.

Possible mechanisms for the occurrence of pain in the distal sensory diabetic polyneuropathy have been discussed by Thomas and Scadding (1987). Ectopic impulses arising in regenerating small myelinated and unmyelinated axons have been suggested as being responsible (Asbury and Fields, 1984). We have previously documented an excess of regenerating myelinated axon sprouts in a case of acute painful diabetic neuropathy (Llewelyn *et al.*, 1986), included as Case 17 in this study. From our present data, the number of RMFs or regenerative clusters alone does not correlate with painful symptoms. Nerve regeneration in man is usually a painless phenomenon and if it does contribute to pain in diabetic neuropathy, then it has to be that other as yet unidentified factors, either local or distant, are influential in modifying their activity.

Dyck *et al.* (1976), from observations on sural nerve biopsies from a series of patients with neuropathies from a variety of causes, concluded that the occurrence of pain was more often correlated with active fibre breakdown than with other types of pathological change. In the present biopsies, including those from Groups 2 and 3 in which pain was a prominent symptom, evidence of active myelinated fibre breakdown was rare, both on light and electron microscopy of transverse sections and in teased fibre preparations.

Excluding the occurrence of pain related to involvement of nervi nervorum, the presence of spontaneous pain in neuropathies is, in general, related to damage to small fibres (Thomas, 1979) but the precise mechanisms remain elusive. Hyperalgesia may result both from sensitization of C fibre nociceptors (Cline *et al.*, 1989) or from pathological changes in large diameter myelinated fibres (Campbell *et al.*, 1988).

ACKNOWLEDGEMENTS

J.G.L. was a Pfizer Research Fellow during part of this study The electron microscope was kindly provided by the Medical Research Council and the image analysis equipment by grants from Ciba-Geigy Ltd, Basel, and the London University Central Research Fund We would like to thank Dr J. Payan for undertaking the nerve conduction studies on the patients from King's College Hospital. The technical help of Jane Workman is gratefully acknowledged, as is the secretarial assistance of Kieran Price.

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(*Received February 7, 1990. Revised May 25, 1990. Accepted May 30, 1990*)

ADDENDUM

Since this paper was accepted, a paper has appeared by Sharma *et al.* (1990) that reports a morphometric analysis on sural nerve biopsy specimens from 4 patients with nonpainful neuropathy, 6 with chronic painful neuropathy and 6 with recurrent foot ulceration. No evidence was found to suggest that selective loss of small myelinated or unmyelinated axons or regenerative clusters were related to the occurrence of pain.

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DOUBLE DISSOCIATION OF SHORT-TERM AND LONG-TERM MEMORY FOR NONVERBAL MATERIAL IN PARKINSON'S DISEASE AND GLOBAL AMNESIA

A FURTHER ANALYSIS

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SUMMARY

The traditional concept of memory disorder is deficiency of the long-term (LTM) but not short-term (STM) component of memory. STM impairment with LTM sparing is seldom reported. The present study investigated STM and LTM for nonverbal material in three neurological conditions associated with memory impairment: bilateral medial temporal lobe lesions (patient H.M.), Parkinson's disease (PD) and Alzheimer's disease (AD). Subjects received 3 tests of nonverbal memory: forward block span, immediate and delayed recall of the Wechsler Memory Scale drawings, and immediate and delayed recognition of abstract designs. Compared with the normal control group, the patient groups displayed different patterns of sparing and loss of the two components of memory: in PD, only STM was impaired; in medial temporal lobe amnesia, only LTM was impaired; and in AD, STM and LTM were both impaired. The contrasting patterns of sparing and loss of STM and LTM in PD and global amnesia were present for both recognition and recall. These results provide evidence that STM and LTM are dissociable processes and are served by separate neurological systems: STM depends upon intact corticostriatal systems, whereas LTM depends upon intact medial temporal lobe systems.

INTRODUCTION

Global amnesia commonly comprises impaired long-term memory (LTM) for new events and facts, regardless of class of material or modality of reception, but spared short-term memory (STM) (e.g., Milner, 1962; Baddeley and Warrington, 1970). This pattern of sparing and loss provides the traditional model of memory impairment. Strongest evidence for this duality of memory for facts comes from studies of patients with bilateral medial temporal lobe resection (Scoville and Milner, 1957; Milner, 1962) and patients with alcoholic Korsakoff's syndrome, which is marked by diencephalic pathology (Victor *et al.*, 1971; Mair *et al.* 1979). The functional impairment may arise from disruption of consolidation of labile STM traces into more stable LTM (Milner, 1962), or from impairment in retrieving memories from LTM storage (Warrington and Weiskrantz, 1970).

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Viability of the STM-LTM dichotomy rests on the demonstration of the double dissociation of sparing and loss (Teuber, 1955) of the two memory systems in different aetiologies of memory impairment (Warrington, 1982). Evidence of STM impairment with LTM sparing provides such a dissociation and stems from neuropsychological and electrophysiological studies.

Lesion studies suggest two possible substrates of STM: frontal and parietal lobe systems. In studies by Warrington and her colleagues (Warrington and Shallice, 1969, 1972; Shallice and Warrington, 1970; Warrington *et al.*, 1971; Warrington, 1982; McCarthy and Warrington, 1987*a*, *b*), patients with left posterior parietal lesions who had been identified on the basis of impaired digit span had an STM impairment for auditory verbal information. In a similar group of patients, Starr and Barrett (1987) found a reduced amplitude of the P300 potential in response to auditory but not visual material and interpreted this observation as a deficit in auditory STM. As with the evidence from the temporal lobe studies, these phenomena may be more intimately connected with sensory or perceptual than with memory processes, in that observation of an STM deficit may require the presence of a language deficit. Support for a frontally-based STM system comes from two additional lesion studies. Lewinsohn *et al.* (1972) demonstrated that impaired STM was associated with lesions anterior to the central sulcus but not lesions located more posteriorly. Modality-specific STM deficits included recall of auditory and visual, but not kinaesthetic, information; no material-specific impairment related to side of lesion was observed. Psychiatric patients treated with leucotomy, involving the orbitofrontal cortex, performed normally on the Wechsler Memory Scale but were impaired on the STM distractor test (Stuss *et al.*, 1982; Stuss and Benson, 1987); and, in a study by Kapur (1988), patients with frontal lobe lesions had an STM, but not an LTM deficit, whereas patients with tumours of the third ventricle showed the opposite pattern of sparing and loss.

Transient STM deficits were elicited in a series of electrostimulation studies of neurosurgical patients with intractable pain or epilepsy. An STM deficit for verbal material occurred with stimulation of the left, but not right, cingulum, whereas a nonverbal memory deficit accompanied stimulation of the right, but not left, cingulum (Fedio and Ommaya, 1970; Ommaya and Fedio, 1972). A further dissociation in the study demonstrated the selectivity of the STM deficit: cingulate stimulation interrupted STM but not LTM, whereas hippocampal stimulation had the opposite effect. In another electrostimulation study, left pulvinar stimulation disrupted verbal STM but stimulation of the right pulvinar, left lateral thalamus, or left or right temporal white matter did not (Ojemann and Fedio, 1968). As noted by Ojemann and Fedio, the pulvinar projects to the posterior temporoparietal cortex, the site of the lesions in patients with STM deficits reported by Warrington and colleagues. Thus material-specific deficits in STM produced by unilateral pulvinar stimulation may be due to disruption of connections between these subcortical and cortical regions.

An additional source of support showing that impaired STM can occur with spared LTM comes from studies of Parkinson's disease (PD) (Tweedy *et al.*, 1982; Taylor *et al.*, 1986; O'Donnell *et al.*, 1987). These studies, however, did not include a pathological control group, which could have provided evidence for selectivity of the deficit through the method of double dissociation (Teuber, 1955). In a comparison between PD and AD, a pathological control group, patients with PD were impaired in word and design recognition after short, but not long, retention intervals; by contrast, patients with AD showed the opposite pattern of performance (Sagar *et al.*, 1988; Sullivan and Sagar, 1989). In these studies, the STM impairment in PD was attributed to frontal lobe dysfunction arising from disruption of basal ganglia/frontal lobe circuitry.

The present study adds evidence to the STM-LTM dichotomy by showing a double dissociation of functions when PD patients are compared with the patient H.M., who has medial temporal-lobe amnesia (Milner, 1958; Corkin, 1984). Although these data have been published separately elsewhere (Sullivan and Sagar, 1989; Sullivan *et al.*, 1989; Sagar *et al.*, 1990), this paper presents the only comparison of the three patient groups. This comparison was presented at the 16th annual meeting of the International Neuropsychological Society (Sullivan and Sagar, 1988).

METHODS

Subjects

The participants included 15 normal control (NC) subjects, 14 PD patients, 15 AD patients and the amnesic patient H.M. PD and AD patients were drawn from the Movement Disorders Unit and the Memory Disorders Unit of the Massachusetts General Hospital. NC subjects were spouses of the PD or AD patients. As described previously (Sullivan and Sagar, 1989), the diagnosis of AD was based on strict inclusion and exclusion criteria (McKhann *et al.*, 1984; Khachaturian, 1985). The diagnosis of PD was based on the presence of 2 of the 3 cardinal signs: akinesia, rigidity and extrapyramidal rest tremor. All patients were ambulant and free from psychiatric disease. All PD patients were treated with antiparkinsonian medication (in the majority of cases a levodopa preparation combined with carbidopa), and responded favourably. Hoehn and Yahr (1967) ratings of the PD patients ranged from stage 2 to 4 in 13 patients; 1 other PD patient was in stage 1 at the time of testing and showed left-sided signs, which later became bilateral. Neither patient group was treated with any primarily psychoactive medication. Patients were not selected for study on the basis of behavioural criteria or treatment regime. The groups were matched for age and years of education but the PD group had a higher male-to-female sex ratio than the other two groups (Table 1). The PD group had a longer mean duration of disease than the AD group.

H.M. had undergone bilateral resection of medial temporal-lobe structures for relief of intractable epilepsy 31 yrs before this testing. The lesion included the hippocampus (the anterior 8 cm), hippocampal gyrus, amygdala and uncus (Scoville and Milner, 1957).

Informed consent for neuropsychological testing was obtained from all subjects.

Wechsler Memory Scale Quotients (MQ), WAIS-R Vocabulary scaled scores and Blessed Dementia Scale (BDS) (Blessed *et al.*, 1968) scores provided general indices of cognitive function. These results appear in Table 2. The mean MQ of the NC group was higher than that of the PD group, which in turn was higher than that of the AD group (F(2,38) = 37.007, $P < 0.001$; Fisher tests for all comparisons,

TABLE 1 CHARACTERISTICS OF SUBJECT GROUPS MEANS AND RANGES

Group	*No of cases (M, F)*	*Age (yrs)*	*Education (yrs)*	*Disease or lesion duration (yrs)*
Control	7, 8	63.1 (54–77)	13.7 (10–19)	Not applicable
Parkinson	12, 2	66.6 (53–80)	15.6 (8–20)	6.9 (1–13)
Alzheimer	7, 8	63.5 (53–76)	14.9 (12–20)	3.2 (2–7)
H.M.	1	58.0	12.0	31.0

TABLE 2 PERFORMANCE OF TESTS OF OVERALL MEMORY, LANGUAGE AND MENTAL STATUS MEANS, SEMs AND RANGES

Group	*Memory Quotient (max. = 143)*	*Vocabulary scaled score (max = 19)*	*Blessed Dementia Scale score* (max = 37)*
Control	119.4	10.7	0.3
	3.61	0.80	0.16
	(93–143)	(7–17)	(0–2)
Parkinson	96.5	11.4	3.3
	4.61	1.00	0.95
	(67–126)	(5–18)	(0–12)
Alzheimer	74.7	8.3	9.1
	3.23	0.43	0.73
	(60–101)	(6–11)	(5–16)
H.M.	64.0	9.0	Not applicable

* Memory and orientation section.

alpha < 0.05). H.M.'s MQ was the lowest of all and was 3 SDs from the mean of published norms. The Vocabulary scaled scores of the PD group were better than those of the AD group ($F(2,39) = 4.338$, $P < 0.02$); the scores of the NC group did not differ significantly from PD or AD groups. H.M.'s Vocabulary score was within the average range. On the memory and orientation section of the BDS, higher scores indicate poor performance. The NC group performed the best, followed by the PD group and then the AD group ($F(2,39) = 44.68$, $P < 0.001$); H.M.'s aetiology made it inappropriate to administer him the BDS. The only behavioural inclusion criterion for patients was that they were able to perform the tests; no NC subject was included whose BDS score exceeded 2.

Procedure

All participants received 3 tests of nonverbal memory: forward block span, recall of Wechsler Memory Scale (WMS) drawings and recognition of nonverbal material. *Forward block span* is a test of immediate nonverbal memory (Milner, 1971; Corsi, 1972). In this test, the examiner touched a series of 3 to 8 of 9 black blocks affixed in an unsystematic array to a blackboard; the subject attempted to touch the same blocks in the presented order. *Recall of the WMS drawings* was done twice. immediate recall followed a 10 s viewing of the drawings (a test of STM), and delayed recall followed unannounced 1 h later (a test of LTM). Two PD and 5 AD patients did not receive the span test, and 1 NC, 1 PD, and 3 AD subjects did not receive the drawing recall test. The *recognition test* used as stimuli swatches of abstract wallpaper designs glued to 5-in. × 8-in. index cards. The designs were presented about 1 every 2 s. At various times throughout the stimulus series, recognition tests were given. For each recognition test, a subject was required to point to 1 of 2 simultaneously displayed designs, indicating which had been seen 1, 50, 100 or 150 cards earlier in the stimulus series; the incorrect design was a foil and had never appeared in the stimulus series. The original test used 9 stimulus-test intervals (1, 3, 6, 10, 15, 25, 50, 100 and 150) and assessed recency discrimination as well as item recognition (Sagar *et al.*, 1988; Sullivan and Sagar, 1989). Five tests were given at each of the 4 stimulus-test intervals. The test at the interval of 1 tapped STM; the index of LTM was the combined tests at intervals of 50, 100 and 150. Data represented from this test are drawn from the studies by Sagar *et al.* (1990) and Sullivan and Sagar (1989).

RESULTS

Analysis of the data was performed with one-way and two-way repeated measures analyses of variance (ANOVA). Post hoc comparisons were based on Fisher tests (alpha = 0.05) and t tests. All probability levels reported are based on two-tailed tests of significance. H.M.'s scores were compared with the normal range, SD of the normal mean and 95% confidence intervals derived from NC, PD and AD data separately (Table 3); because of skewing of some data sets, the 95% confidence limits did not always bear a predicted relationship to the SD. Accordingly, all comparisons are presented. H.M.'s results from the WMS and block span are similar to those reported by Corkin (1984; Corkin *et al.*, 1985).

Block span

The PD and AD groups had impaired forward block spans (F(2,34) = 7.397, $P <$ 0.003) and did not differ from each other (fig. 1). H.M.'s block span was within the normal range and within 1 SD of the NC mean. His span fell just below the lower confidence limit of the NC group but above the upper confidence limit of the PD group (Table 3).

TABLE 3 95% CONFIDENCE INTERVALS OF TEST SCORES FOR 3 GROUPS AND TEST SCORES FOR PATIENT H.M.

		Design Recognition		*Drawing Recall*	
Group	*Block Span*	*Immediate*	*Delayed*	*Immediate*	*Delayed*
Control	5.3–6.1	5.8–9.2	2.9–6.5	68.9–91.1	71.8–84.7
Parkinson	4.2–4.8	1.7–5.4	1.1–4.9	49.0–68.1	64.6–84.1
Alzheimer	3.0–5.4	0.2–3.9	0–1.2	50.0–78.0	58.1–71.8
H.M.	5.0	4.0	0.0	80.0	40.0

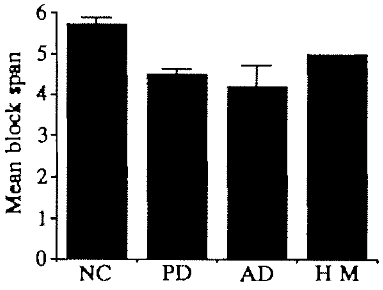

FIG. 1. Immediate memory span for blocks. The maximum score is 8 The PD and AD groups showed impaired performance H.M.'s span was within the normal range and within 1 SD of the normal mean.

Recall of drawings

These analyses are based upon data reported by Corkin *et al.* (1985) and Sullivan *et al.* (1989). A two-way ANOVA of 3 groups by 2 conditions (immediate or delayed) revealed significant main effects of group (F(2,36) = 12.589, $P < 0.001$) and condition (F(1,36) = 19.912, $P < 0.001$) and a trend towards an interaction (F(2,36) = 2.778, $P < 0.08$). Three two-way ANOVAs were performed in order to observe the pattern of results of group pairs. The results for the AD vs NC groups and for the AD vs PD groups ANOVAs were the same, in that the group (AD vs NC: F(1,26) = 25.205, $P < 0.0001$; AD vs PD: F(1,22) = 4.423, $P < 0.05$) and the condition (AD vs NC: F(1,26) = 20.797, $P < 0.0001$; AD vs PD: F(1,22) = 5.535, $P < 0.03$) effects, but not the interaction (AD vs NC: F(1,26) = 1.679, $P > 0.02$; AD vs PD: F(1,22)

= 1.155, $P > 0.2$), were significant. By contrast, the interaction for the PD vs NC groups was significant (F(1,24) = 5.334, $P < 0.03$) as were the main effects of group (F(1,24) = 6.546, $P < 0.02$) and condition (F(1,24) = 14.655, $P < 0.001$), indicating that the adverse effect of the delay was greater in the NC than the PD group.

For immediate recall of WMS drawings, the PD and AD groups were impaired (one-way ANOVA, F(2,36) = 12.298, $P < 0.001$); H.M.'s score fell within the normal range and within 1 SD of the normal mean but below the lower confidence limits of the NC group; his score was above the upper confidence limit of the AD group (fig. 2, Table 3). After the 1 h delay, the scores of the NC (t(14) = 4.01, $P < 0.002$) and AD (t(12) = 2.309, $P < 0.04$) groups and patient H.M. declined, whereas those of the PD group remained similar to its immediate scores (t(10) = 0.875). For delayed recall (one-way ANOVA, F(2,37) = 9.494, $P < 0.01$), the PD group was superior to the AD group ($P < 0.002$) and H.M. and equivalent to the NC group. Thus memory deficits of the PD group were all evident in the short-term and did not deteriorate further after a delay. Moreover, the poorer delayed recall scores of H.M. and the AD group, compared with immediate and delayed recall of the PD group, indicate that the PD scores were not at floor level.

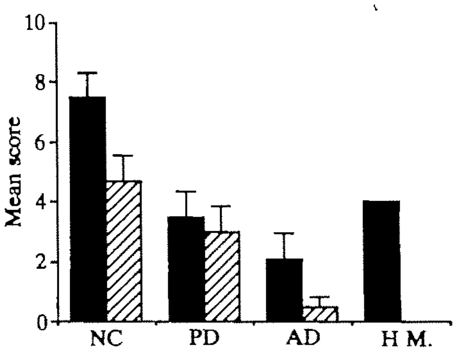

FIG. 2. Recall of the Wechsler Memory Scale drawings. The maximum score for each test session is 14. The NC and AD groups and patient H M showed the same pattern of performance immediate recall (filled columns) was significantly superior to delayed recall (hatched columns) By contrast, immediate and delayed recall scores of the PD group were equivalent.

Recognition of designs

These analyses are based upon data reported by Sagar *et al.* (1990) and Sullivan and Sagar (1989). Only the group factor was significant in the three-group, two-way ANOVA (F(2,41) = 5.095, $P = 0.011$); neither the task factor (F(1,41) = 1.886, $P < 0.18$) nor the group-by-task interaction was significant (F(2,41) = 2.447, $P < 0.10$).

Performance by the NC group was virtually the same under STM and LTM conditions (t(14) = 0.38) (fig. 3). The AD group showed the normal pattern of performance (STM vs LTM: t(14) = −0.148) but at a significantly lower level. This observation was confirmed with a two-way ANOVA for the NC and AD scores, showing that only the group factor was significant (F(1,28) = 7.412, $P = 0.011$; for short vs long delay: F(1,28) = 0.011; for interaction, F(1,28) = 0.1199). By contrast, H.M.'s immediate recognition was identical to the mean of the NC group (Table 3) but fell to chance level under LTM conditions. The PD group displayed yet another pattern: for STM recognition, the scores of the PD group were significantly worse than those of the NC group and H.M. but not the AD group; for LTM recognition, the PD group's score rose to the level of the NC group and surpassed the AD level (for PD, STM vs LTM: $t(12) = 2.309$, $P < 0.04$) (fig. 3). While a two-way ANOVA comparing the PD and AD groups showed no significant effects (group: $F(1,27) = 0.158$; short vs long delay: $F(1,27) = 3.045$, $P < 0.10$; interaction $F(1,27) = 2.554$, $P < 0.13$), the PD-NC comparisons were significant for group ($F(1,27) = 7.639$, $P = 0.01$) and the group-by-interval interaction ($F(1,27) = 4.592$, $P < 0.05$), but not for interval ($F(1,27) = 2.68$, $P > 0.10$). The normal recognition scores of the PD group at the longer intervals indicate that their impaired performance at the short interval is not solely attributable to impairment in visual perception or attention.

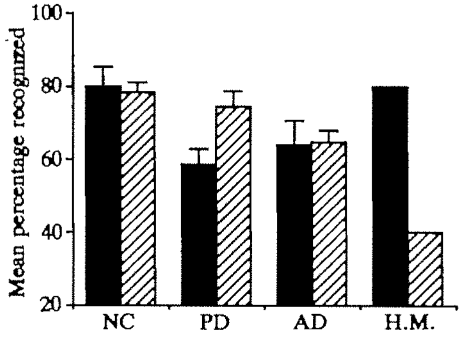

FIG. 3. Recognition of abstract designs. Chance performance is 50%. The NC and AD groups showed the same pattern of performance, in that the immediate (filled columns) and delayed (hatched columns) recognition levels within these two groups were equivalent, although the AD group was impaired Patient H.M 's performance was typically amnesic, with immediate superior to delayed recognition. The PD group showed a pattern of performance opposite to the amnesic pattern.

DISCUSSION

The opposite patterns of sparing and loss of STM and LTM in global amnesia and PD occurred for recognition and recall of newly-acquired nonverbal, visual material. These results provide evidence that STM and LTM are dissociable processes and are served by separate neurological systems.

STM-LTM functional differences

Traditionally, the STM-LTM dichotomy has been accommodated within the information processing model (e.g., Broadbent, 1958; Waugh and Norman, 1965; Atkinson and Shiffrin, 1968; for review, Baddeley, 1986). This model, derived from experiments with normal subjects, assumes that stimuli, once identified or encoded, are supported temporarily by STM. If the information held in STM is not forgotton by interference or elapse of time, it can progress to the large capacity, relatively permanent store of LTM. Dual process theorists suggest that the rapidly declining arm of the initial part of a typical forgetting function for verbal and nonverbal material in recall paradigms reflects STM, and the flat asymptotic function reflects LTM. In nonverbal recognition paradigms, little forgetting may occur even after long retention intervals (e.g., Paivio, 1971; Sullivan and Sagar, 1989). A strong test of these theories arises from studies of neurological patients with known CNS dysfunction (cf, Mayes, 1988). In particular, selective disruption of each hypothesized memory component by different lesions would provide support for the models.

In the present study, immediate recall of the drawings was impaired in PD, thus replicating a finding by Bowen *et al.* (1972), but delayed recall was not. The forgetting curve, in this instance, was flat, indicating that whatever was remembered initially was retained far beyond the life of STM. Thus it appears that encoding, storage and/or retrieval in PD may be fundamentally intact when the processing capacity of one or more STM operations is reduced. Consolidation, as defined by Milner (1962), can be normal in PD but is severely impaired in global amnesia and, presumably, progressively impaired in AD.

In order to account for preservation of memory over time, the information processing model must make assumptions about its component operations: LTM depends on adequate STM processing; furthermore, recall and recognition require encoding and retrieval operations, which develop, at least to some extent, in parallel. Three scenarios of STM impairment emerge from these assumptions: (1) encoding is slowed or subject to poor strategy and retrieval is intact; (2) encoding is intact and retrieval is slow or subject to poor strategy; (3) encoding as well as retrieval mechanisms are slow or poor.

Although the information processing model can account for a STM deficit with LTM sparing, another model may explain the dissociation more parsimoniously. The alternative explanation is based on a version of the reverberating circuit model (Hebb, 1949; P.M. Milner, 1989) and models suggested by others (e.g., Peterson, 1966; Wickelgren, 1968; Shallice and Warrington, 1970), which consider STM and LTM as separate and parallel processes. Traces are laid down and evolve in parallel through both memory processes. STM produces short-lived traces that are quickly forgotten as compared with the potentially permanent traces produced by LTM. The likelihood of retrieving a particular memory trace from one system over the other depends upon the time and activity between stimulus reception and retrieval. Thus when a memory is retrieved shortly after reception, the STM trace is invoked because it develops faster and is more viable in the short span than the LTM trace, which is still evolving. Within the constructs of this model, traces supported by LTM need not be preprocessed by STM; moreover, the degree of trace stability depends on the memory system from which information is retrieved. Thus the parallel processing models predict independence of the two memory systems, a conclusion supported by the results of the present study.

The selective disruption of one of several related cognitive processes is evidence for the relative independence of the process compared with other processes within its domain. Importantly, in the present example, the STM deficit does not produce either a rise or a decline in LTM. Instead, STM and LTM are hypothesized to be dissociable, relatively independent systems whereby a deficit in one does not necessarily cause a deficit in the other. Thus the modified Hebbian parallel model of memory more suitably fits the data of the present study than does the information processing model.

STM in PD

The PD group was deficient in nonverbal short-term recognition when compared with the NC group and patient H.M. As previously discussed (Sullivan and Sagar, 1989), this finding is in apparent contradiction to that of Flowers *et al.* (1984) who reported that nonverbal recognition was spared in their PD patients. In the Flowers *et al.* study, however, long-term, but not short-term, recognition memory was tested. In their study as well as in this one, long-term recognition memory for nonverbal material was indeed intact in PD, but we found, in addition, that STM was defective.

The STM deficit with relative LTM sparing may provide experimental evidence for the clinical manifestation of bradyphrenia in PD. Bradyphrenia describes cognitive performance that appears slow but is accurate if the subject is given sufficient time to respond, although the underlying deficit need not involve slowing of cognitive processes. This claim was made previously as an interpretation of an STM deficit for word recognition (Sagar *et al.*, 1988). The concept of an STM deficit as the basis of bradyphrenia predicts that a similar deficit should occur for recall; impaired recall of WMS designs by the PD group of the present study fulfills this prediction.

The adverse effects of dementia per se cannot account for clinical bradyphrenia or the experimental STM deficit because, in the recognition test, the AD patients were severely impaired in LTM despite their relatively mild STM deficit. Additionally, impaired performance at the stimulus test interval of 1 occurred even in a subgroup of PD patients whose BDS scores were within the normal range (Sullivan and Sagar, 1989).

The findings of selective STM deficit with LTM sparing do not exclude the possibility of an additional LTM deficit in PD under some conditions. Indeed, such observations have been made (Sagar *et al.*, 1988; Levin *et al.*, 1989). However, the results of this study provide clear evidence for potential dissociation of STM and LTM when PD and global amnesia are compared on the same tasks.

STM in global amnesia

When compared with the NC group, H.M.'s level of STM functioning was marginal on 2 tests. On block span, his score was at the low end of the range for normal subjects of this study and at the low end of the theoretical, immediate memory limit as predicted by Miller's (1956) 'magical' number seven, plus or minus two. On the drawing test, H.M.'s immediate recall was just outside 1 SD of the NC group mean and below the lower NC confidence limit; this result is not surprising because the number of elements to be remembered from the drawings exceeds the limits of STM capacity. H.M.'s immediate recognition score, however, equalled that of the NC group. In general, H.M.'s performance was characterized by marked LTM loss and STM sparing and showed a distinctly different pattern from the PD group.

Implication for a neurological substrate of STM

The double dissociation of STM and LTM in PD and the patient H.M. provides evidence that STM is served by neurological systems that are involved in PD but is independent of the medial temporal lobes, which were resected in H.M. Additionally, LTM is severely disrupted by hippocampal lesions, which are present in H.M. as well as AD (Blessed *et al.*, 1968; Ball, 1977; Kemper, 1984), but is not so dependent on systems defective in PD (reviewed by Sagar and Sullivan, 1988). The LTM link with the medial temporal lobes is well established (e.g., Milner, 1962; Corkin, 1984), but specifying a neurological basis for STM has proved to be more elusive. Neural systems that have been claimed to support STM are the left parietal lobe (Warrington *et al.*, 1971) and its connections from the pulvinar (Ojemann and Fedio, 1968), and the frontal lobes (Lewinsohn *et al.*, 1972; Stuss and Benson, 1987; Kapur, 1988) and their connections with the cingulum (Fedio and Ommaya, 1970; Ommaya and Fedio, 1972). Arguments for a frontal basis for STM date from studies of monkeys with frontal lobe lesions which were impaired in a delayed alternation task (Jacobsen, 1935, 1936). The original claim was that the frontal lobe lesion interfered with memory for the prior location of the bait. The memory hypothesis was later refuted, however, and the deficit was attributed to nonmnemonic factors, including distractibility and reduced ability to suppress competing alternatives (review by Stuss and Benson, 1984; Fuster, 1985).

The observation of verbal (Sagar *et al.*, 1988) and nonverbal (this study) STM deficits in PD suggests that STM depends on a frontal-basal ganglia system rather than the parietal lobes for the following reasons. First, the evidence for linking STM to parietal lobe function was based on a material-specific deficit for verbal information only (e.g., Warrington, 1982). By contrast, in our studies STM deficits included both verbal and nonverbal material and thus imply bilateral involvement. Secondly, the primary pathology of PD involves lesions of the basal ganglia and degeneration of the dopaminergic system. The basal ganglia have multiple bilateral projections to the frontal lobes but fewer to the postcentral cortex, including the parietal lobes (Nauta and Domesick, 1978, 1984; Graybiel, 1984; Alexander *et al.*, 1986).

The prefrontal cortex comprises a host of discrete neuroanatomical, neurochemical and neuropsychological systems (for review, *see Trends in NeuroSciences*, **77**, November 1984). Anatomical and functional dissociations among specific regions of the frontal cortex are well known in primates, including man. For example, patients with dorsolateral frontal lesions are impaired on the Wisconsin Card Sorting Test (Milner, 1964) and recency discrimination (Corsi, 1972; Milner *et al.*, 1985), whereas patients with orbitofrontal lesions tend to perform poorly on the STM distractor task (Stuss and Benson, 1987) and symbolic fluency (Milner, 1964; Jones-Gotman and Milner, 1977). Despite the possibility that STM also depends on frontal lobe integrity, the specific frontal system responsible for STM function remains unknown. The impairment of leucotomy patients on the STM distractor task, however, strongly implicates the orbitofrontal cortex in STM function (Stuss *et al.*, 1982). By contrast, the deficit of PD patients on the Wisconsin Card Sorting Test has been attributed to deficient STM (Bowen *et al.*, 1975) and, by implication, the dorsolateral prefrontal cortex (Milner, 1964; Weinberger *et al.*, 1986). Not all patients with frontal lobe pathology have STM deficits, however; Ghent *et al.* (1962) reported that World War II veterans with penetrating frontal lobe injuries showed no impairment in verbal or nonverbal STM. Similarly in Korsakoff's syndrome, STM is spared in the face of frontal lobe atrophy and severe LTM deficits (Lishman, 1978; Butters and Cermak, 1980; Shimamura *et al.*, 1988).

These contradictory points of evidence for a neurological substrate of STM need to be resolved. A suggestion of a resolution comes from a neuroanatomical study (Selemon and Goldman-Rakic, 1988) presenting data that link two proposed substrates of STM despite their spatial disparity. The dorsolateral prefrontal cortex and the posterior parietal cortex share multiple projection sites, several of which are implicated in PD. Whether interruption of any of these common pathways can influence behaviour, such as STM, to the extent that cortical lesions do, remains to be established. A study of the behavioural consequences of specific subcorticofrontal neural and neurochemical pathology in PD may lead to an answer to this query and provide a functional basis for STM that supplements the traditional anatomical evidence derived from focal cortical lesions.

ACKNOWLEDGEMENTS

We thank Dr Brenda Milner in affording us the privilege to study patient H.M.; Dr Suzanne Corkin for support during testing and for comments on this manuscript; and Dr John H. Growdon for allowing us to test his patients with Alzheimer's disease and patients with Parkinson's disease. We also thank Rae Ann Clegg, Allison Feeley, Kathleen Coffin and Marguerite Randolf for help with scheduling and testing.

This research was supported by grants from the National Institutes of Health, USA, to the Massachusetts Institute of Technology (RR 00088, MH 32724 and MH 2433) and to Harvard Medical School at the Massachusetts General Hospital (P50-AG05134) and by the Oxford Regional Health Authority, UK. Support during manuscript preparation was provided by a grant from the National Institutes of Health (MH 30854) and (MH40041), Medical Research Service of the Department of Veterans Affairs, Special Trustees of the Former United Sheffield Hospitals, UK and Roche Products, UK.

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(*Received March 31, 1989. Revised May 22, 1990. Accepted May 30, 1990*)

Brain (1991), **114**, 907–913

DIFFERENTIAL EFFECT OF COMPRESSION-ISCHAEMIA BLOCK ON WARM SENSATION AND HEAT-INDUCED PAIN

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SUMMARY

The effect of compression-ischaemia nerve block on psychophysical thresholds for warm sensation and heat-induced pain was studied on 19 normal human volunteers. Although those two sensory submodalities should be predicted to block simultaneously, based on the fact that both are served by unmeylinated primary afferents, it was actually found that warm sensation was much more vulnerable to compression-ischaemia than heat-induced pain. This is interpreted as resulting from different summation requirements for each of the two sensory modalities; sensation of warmth depends on spatial summation to a larger extent than heat-induced pain. Such differential vulnerability is in line with recent clinical studies reporting deterioration of warm sensation associated with preservation of heat pain in peripheral nerve disorders caused by diabetes, ageing and other neuropathic processes.

INTRODUCTION

Application of a constricting force to a nerve is a time-honoured method for blocking nerve fibres differentially (Bastien and Vulpian, 1855 (direct pressure to several human nerves); Gasser and Erlanger, 1929 (cuff, in vitro, animals); Lewis *et al.*, 1931 (cuff, humans); Zotterman, 1933 (cuff, humans); Gasser, 1935 (cuff, in vivo, cat); Sinclair and Hinshaw, 1950 (cuff, humans, and literature review), and others). The relationship between time to block and the type of nerve fibre subpopulation blocked has been established electrophysiologically. Under direct nerve compression in vitro, fibres of similar conduction velocity stop conducting nerve impulses at the same stage along the course of the block, fast A fibres blocking first and slow C fibres last (Gasser and Erlanger, 1929). Early loss of impulse propagation in myelinated A fibres in experimental animals during direct nerve compression, or whole limb compression-ischaemia blocks was indirectly correlated with human psychophysical studies, revealing corresponding early abolition of perception of tactile and cold stimuli (*see* Sinclair and Hinshaw, 1950). Thus tactile and cold sensations can be accepted as being subserved by myelinated primary afferents, while the residual sensations of warmth and pain are accepted as subserved by unmeylinated fibres, as both disclose similar resistance to block (Lewis *et al.*, 1931; Sinclair and Hinshaw, 1950). Eventually these claimed associations between sensations and fibre types were directly confirmed microneurographically in awake humans (Torebjörk and Hallin, 1973; Mackenzie *et al.*, 1975; Hallin *et al.*, 1982).

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If a compression-ischaemia block were maintained long enough to interfere with conduction of unmyelinated fibres, similar blocking times for warm sensation and heat pain should be anticipated in theory since the corresponding primary afferents conduct at a similar velocity in humans (Konietzny and Hensel, 1975; Torebjörk *et al.*, 1984). Nevertheless, our results show that at a stage when thresholds for warm sensation are significantly elevated, those for heat pain are still unchanged.

METHODS

Sensory testing

Routine clinical sensory examination included (1) evaluation of light touch tested by weak mechanical stimuli applied by stroking with a fine brush; (2) evaluation of mechanical pain, tested by intense mechanical stimuli applied by pin prick; and (3) evaluation of cold-specific sensation tested by surface application of an ice cube. All tests were performed on hairy skin on the dorsum of the hand.

Quantitative testing of thermal-specific and thermal pain submodalities (warm sensation, cold sensation, heat-induced pain and cold-induced pain) was performed by measuring thresholds during delivery of ramps of relative high or low temperature stimuli using the Thermotest (Somedic AB, Stockholm), as described by Fruhstorfer *et al.* (1976). A Peltier type thermode measuring 2.5×5 cm was applied to glabrous skin at the thenar eminence of normal human volunteers. Temperature of the thermode could either rise or fall, at various rates, from the adapting temperature of 32° C, depending on the direction and intensity of the current flow through the Peltier device. The subject held a switch in the free hand, to be pressed at the first sensation of either warmth, cold, heat pain or cold pain. Pressing the switch reversed the temperature of the probe, returning it to the adapting level. An Omniscribe chart recorder (D 5000, Houston Instruments, Austin, Texas) reproduced the results graphically The rates of temperature change were 1.2° C/s for warm sensation and 3° C/s for heat pain.

Compression-ischaemia nerve block

After obtaining baseline threshold measurements, a sphygmomanometer cuff was inflated at upper arm level to a pressure 80–100 mmHg above systolic blood pressure. Hand temperature was monitored on the skin adjacent to the thermode, using a digital thermometer (2100 Tele Thermometer, VSI, Yellow Springs, Ohio). It was maintained within 1° C of baseline temperature by a radiating heat source which counteracted the tendency towards cooling during ischaemia. Compression-ischaemia was sustained for 1 h or until the volunteer requested release due to discomfort or pain underneath the cuff. Thermotest threshold measurements were performed cyclically throughout the test session, supplemented by clinical sensory testing. In addition to signalling thresholds, the subject was instructed to describe verbally sensations evoked by the various stimuli. In order to avoid anticipatory reactions, the sequence of presentation of the thermal stimulations was changed randomly from one cycle to the other. However, the two thermal sensations always preceded the two thermal pains. Since individual measurement times differ, all results were grouped to the nearest 5 min and then averaged. Results pertaining to low temperature stimulation are reported separately (Yarnitsky and Ochoa, 1990*a*).

RESULTS

The study was performed on 19 healthy volunteers, 7 males and 12 females, aged 19–50 (mean 34) yrs, all of whom gave informed consent. The right hand was used in 12 subjects, the left in 7. Four volunteers tolerated the cuff for a full hour while the other 15 failed to endure this length due to local pain at the site of the cuff. Mean cuff time was 43.37±2.36 (range 27–60) min, all results being given as mean±SEM.

The sensation evoked by light brushing of the skin was lost at 21.68±0.99 min of block. Subjects started reporting a change in the sensation induced by pin prick at 20.94±2.53 min. This consisted of transformation from sharp to dull pain in 14, to 'something' in 2 and to no sensation in 1. No change in the sensation induced by pin prick was reported by 2. A mild low temperature stimulus was no longer perceived as cold beyond 21.14±2.39 min by 14 subjects and the ice cube was not perceived as cold beyond 20.36±2.33 min in 13 subjects.

Warm sensation

After a small initial increase in thresholds (from 33.15±0.15 at baseline to 34.01±0.20 at 5 min of block), which could be related to the distraction surrounding cuff inflation (Kojo and Pertovaara, 1986), thresholds for warm sensation increased very gradually over 25 min. By that time a definite increase of threshold was observed, reaching 37.37±0.44° C at 40 min of cuff (fig.). Mean thresholds at 5 and 40 min were significantly different ($P = 0.0016$, Wilcoxon Signed Rank test).

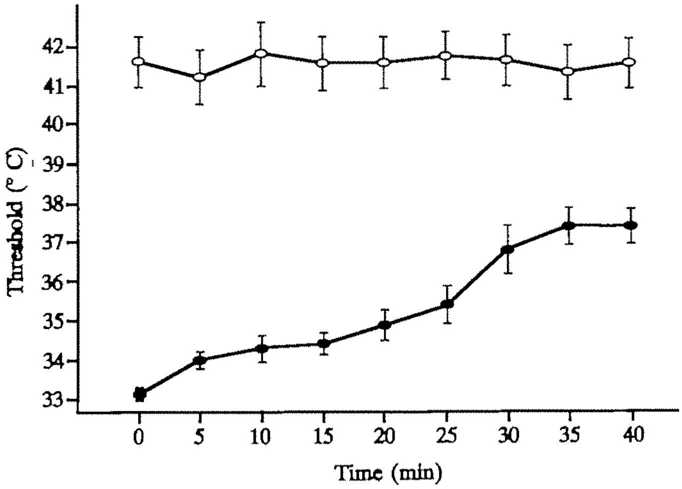

FIG. Mean thresholds (±SEM) for warm sensation (filled circles) and heat pain (open circles) at various times along the course of compression ischaemia block. Note eventual increase in thresholds for warm sensation and unchanged thresholds for heat pain.

The quality of sensation evoked by a mild high temperature stimulus was unchanged in 14 subjects, while in the remaining 5 subjects sensations of 'something', pain, sting, sharp and burning were reported during the block.

Heat pain

Mean thresholds for pain induced by heat were essentially unchanged throughout the test (fig.). Baseline measurement revealed a mean threshold of 41.61±0.63° C. During the cuff session, mean threshold varied between 41.20±0.67 (at 5 min) and 41.73±0.58° C (at 25 min).

The painful sensation reported when delivering a noxious hot stimulus was quite resistant to block. One subject lost the heat quality at 34 min, but kept reporting pain, and another started reporting 'sting' at 23 min. The other 17 subjects kept reporting heat pain throughout the block.

DISCUSSION

The distinct sensations of warmth and delayed pain have traditionally been correlated with conduction in unmyelinated primary afferents (*see* Sinclair and Hinshaw, 1950, for a review). Indirect evidence supporting this concept has been raised through experimental differential nerve blocks. Direct nerve compression and whole limb compression-ischaemia blocks applied to humans result in abolition of the sensations of touch and cold very close in time to the abolition of impulse propagation in myelinated fibres under similar block in animals. Beyond this stage of block, warmth and pain are still perceived while the only available afferents in the animal model are unmyelinated fibres (Lewis *et al.*, 1931; Gasser, 1935; Sinclair and Hinshaw, 1950). Dyck and Lambert contributed clinical-pathological evidence supporting these correlations by showing (1) striking loss of large myelinated fibres in disease states that feature loss of perception of light touch (1968), and (2) loss of unmyelinated fibres in amyloid neuropathy in patients with loss of temperature and pain sensation (1969). Through the use of microneurography, relationships between sensory submodality and afferent fibre type have been confirmed directly in humans during differential nerve blocks (Torebjörk and Hallin, 1973; Mackenzie *et al.*, 1975).

Eventually, direct nerve recordings in animals and humans established the subdivision of unmyelinated primary afferents into (1) warm specific units, responding exclusively to mild, nonnoxious temperature elevation (Konietzny and Hensel, 1975; Darian-Smith *et al.*, 1979; Duclaux and Kenshalo, 1980) and (2) polymodal nociceptor specific units that fire in response to intense mechanical and thermal stimuli inclusive of noxious heat (Torebjörk, 1974; Hallin *et al.*, 1982; Van Hees and Gybels, 1981). These two subsets of fibres have similar conduction velocities, and therefore can be taken to have similar axon calibres, although direct morphometric studies of functionally identified unmyelinated afferents in humans remain to be obtained.

Surprisingly, dissociated clinical behaviour has been reported recently for the sensations related to these two groups of unmyelinated primary afferents. The ability to sense warm stimuli may be significantly reduced while no change is detected in the ability to sense heat pain stimuli in the elderly (Kenshalo, 1986; Mitchell and Schady, 1988), in diabetic patients (Claus *et al.*, 1987; Ziegler *et al.*, 1988*a*, *b*; Navarro *et al.*, 1989) and in various types of neuropathic patients (J. Ochoa and U. Lindblom, unpublished observations). Assuming that primary afferents of similar structural properties are bound to be equally affected by systemic degenerative processes, such dissociated impairment of function calls for explanation.

One possibility assumes different central spatial summation requirements for the sensory channels concerned, such that during progressive and equivalent depopulation of functioning primary afferents a point in time comes when weak summation jeopardizes the sensation that requires it most. Several studies have been conducted on the spatial summation requirements for sensations evoked by high temperature stimuli. Lele (1954), Kenshalo *et al.* (1967), Stevens *et al.* (1974) and Kojo and Pertovaara (1987), all demonstrated strong dependence of warm-specific perception thresholds on the area of stimulation; thresholds decreased as stimulus area increased. The effect of stimulus area on heat pain thresholds is less clear. Machet-Pietropaoli and Chery-Croze (1979) and Kojo and Pertovaara (1987) found a similar inverse relation between heat pain thresholds and stimulus area, but Greene and Hardy (1958) found heat pain thresholds to be only minimally affected by stimulus area, and Stevens *et al.* (1974) also found the stimulus area factor to be negligible for hot stimuli at pain threshold intensity. More recently it has been shown that selective intraneural stimulation of a few unmyelinated nociceptors can be sufficient to induce a delayed burning pain (Ochoa and Torebjörk, 1990), whereas warm sensation cannot be induced by weak intraneural microstimulation, implying a significant spatial summation requirement (*see* Ochoa, 1984).

Another possible explanation for the dissociated behaviour of warm sensation versus heat pain might be a relatively smaller number of warm-specific units, compared with C nociceptors (Handwerker and Neher, 1976; Hallin *et al.*, 1982; Saumet *et al.*, 1985). Even if the two submodalities had similar spatial summation requirements, such that equivalent depopulation of their primary afferents (by disease or experimental block) would cause equivalent elevation of thresholds, the numerically underrepresented warm-specific afferent function would defect earlier below a critical level.

Differences in receptor susceptibility to ischaemia may be a contributing factor in determining the response to block. While resistance of rabbit polymodal nociceptors to a short period of ischaemia was demonstrated by Lynn (1979), the behaviour of warm-specific units has not, to our knowledge, been tested under those circumstances. This possibility remains open.

Although there exists evidence for participation of small myelinated A-delta fibres, in addition to unmyelinated nerve fibres, in conduction of impulses induced by a noxious hot stimulus (Lewis and Pochin, 1937; Price *et al.*, 1977; Campbell and LaMotte, 1983), for all practical purposes the sensation of heat pain, as probed in the present study, is a C fibre conducted submodality. Indeed, we tested thermal sensations in glabrous skin quantitatively, where both microneurographic (Torebjörk and Ochoa, unpublished) and psychophysical (Campbell and LaMotte, 1983) data fail to demonstrate responsiveness of A delta fibres to the stimulus at intensity near heat pain threshold. Moreover, even in hairy skin, at the relatively low rate of temperature rise used to evoke heat pain in the present study, reaction time measurement indirectly indicates peripheral conduction in the unmyelinated fibre range of velocity (Yarnitsky and Ochoa, 1990*b*).

ACKNOWLEDGEMENTS

This study was supported by NIH grants R01 NS 24740 and 24766.

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(Received August 15, 1989. Revised June 5, 1990. Accepted June 9, 1990)

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EXPERIMENTAL VITAMIN E DEFICIENCY IN RATS

MORPHOLOGICAL AND FUNCTIONAL EVIDENCE OF ABNORMAL AXONAL TRANSPORT SECONDARY TO FREE RADICAL DAMAGE

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SUMMARY

Morphological and functional studies have been performed on experimental vitamin E deficient rats. The predominant morphological change was axonal dystrophy and degeneration in the rostral parts of the dorsal columns, particularly in the gracile fasciculi. The dystrophic changes comprised focal axonal swellings containing accumulations of normal and abnormal organelles which included tubulovesicular structures probably derived from the smooth endoplasmic reticulum, mitochondria, dense lamellar bodies, neurofilaments, multifascicular bodies and lysosomes. Similar but lesser changes were observed in distal peripheral nerves. The appearances suggested a disturbance of axonal transport with a defect of 'turnaround' in the distal axons. Studies on the axonal transport of endogenous acetylcholinesterase showed an impairment both of fast anterograde and retrograde transport. The changes were considered to be secondary to the lack of the antioxidant effect of vitamin E as the neurological deficits could be reduced by the concomitant dietary administration of the synthetic antioxidant ethoxyquin and were markedly aggravated by the administration of polyunsaturated fatty acids. It is suggested that the neurological syndrome produced by vitamin E deficiency could be the result of damage to the function of mitochondria and other intra-axonal membranous structures which would interfere both with fast anterograde transport and 'turnaround' and lead to a distal axonal degeneration.

INTRODUCTION

'Vitamin E' is the generic term given to a group of lipid soluble compounds which are derivatives of tocol and tocotrienol, the former having a saturated and the latter an unsaturated side chain. Alpha-tocopherol has the greatest biological activity and accounts for more than 90% of vitamin E in tissues. A prolonged and severe deficiency of vitamin E gives rise to a neurological syndrome characterized by ataxia, tendon areflexia, a 'large fibre' sensory neuropathy and muscle weakness (*see* Harding, 1987). This occurs in abetalipoproteinaemia (Muller *et al.*, 1983), cystic fibrosis (Farrell *et al.*, 1977; Willison *et al.*, 1985), chronic cholestasis in children (Elias *et al.*, 1981; Rosenblum *et al.*, 1981), chronic intestinal malabsorption in adults (Harding *et al.*, 1982) and selective impairment of intestinal absorption of vitamin E (Harding *et al.*, 1985; Sokol *et al.*, 1988). Pathological studies in patients with cystic fibrosis have demonstrated fibre degeneration in the posterior columns of the spinal cord (Geller *et al.*, 1977) and the presence of dystrophic axons similar to those observed in experimental vitamin E deficiency in the gracile nuclei (Sung, 1964; Sung and Stadlan, 1966). It was suggested

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that these changes were related to the deficiency of vitamin E since their incidence declined after the introduction of prophylactic vitamin E therapy (Sung *et al.*, 1980).

A neurological syndrome has been observed in rats with severe and prolonged experimental vitamin E deficiency, comprising muscle weakness, tremor, ataxia and hyperaesthesia (Ringsted, 1935; Luttrell and Mason, 1949; Einarson, 1952; Machlin *et al.*, 1977; Towfighi, 1981). Morphological studies have shown that this is associated with the development of axonal dystrophy in the rostral parts of the dorsal columns and the gracile and cuneate nuclei (particularly the former) with secondary loss of myelin (Pentschew and Schwartz, 1962; Lampert *et al.*, 1964) and accompanying wallerian-like fibre degeneration. Dystrophic changes and fibre degeneration of lesser severity develop distally in lower limb nerves (Nelson *et al.*, 1981; Towfighi, 1981). The morphological changes are those of a central-peripheral distal axonopathy (Spencer and Schaumburg, 1976).

The mechanism of the neurological abnormalities in vitamin E deficiency has not yet been established. In vitro, and probably in vivo, vitamin E acts as an antioxidant. It appears to be the only significant lipid-soluble chain-breaking antioxidant in vivo (Ingold *et al.*, 1987). It is able to terminate free radical-generated chain reactions by scavenging peroxyl radicals formed by the action of oxygen-derived free radicals on unsaturated fatty acids. Vitamin E is therefore likely to be important for the prevention of lipid peroxidation of the polyunsaturated fatty acids (PUFA) of membrane phospholipids (Tappel, 1962, 1965; McCay *et al.*, 1971, 1972). It may also protect other molecules such as membrane proteins from oxidation. In addition it has been suggested that the phytyl side chain of the tocopherol molecule can interact with the arachidonyl fatty acid chains of membrane phospholipids (Diplock and Lucy, 1973) and in this way facilitate molecular packing and the maintenance of membrane stability (Maggio *et al.*, 1977).

Evidence that the neurological sequelae of vitamin E deficiency result from the antioxidant properties of vitamin E was provided by Nelson (1987) who reported that the neuropathological abnormalities could be prevented by the synthetic antioxidants ethoxyquin and promethazine.

The mechanism whereby a deficiency of vitamin E leads to a distal length-related axonopathy has not, however, yet been explained. The accumulation of organelles in the dystrophic axons suggests that a disturbance of axonal transport is involved (Bradley *et al.*, 1986). In this study, we have therefore examined morphometric changes and alterations in fast anterograde and retrograde axonal transport in peripheral nerve in experimental vitamin E deficiency in the rat. In an attempt to investigate whether the antioxidant properties of vitamin E are important, we have assessed the effect of (1) feeding increased amounts of dietary polyunsaturated fatty acids which provide increased quantities of peroxidizable substrate and (2) the administration of the artificial antioxidant, ethoxyquin, which should have a protective effect.

Preliminary accounts of some of the results have been presented in abstract form (Bradley *et al.*, 1986; Southam and Thomas, 1989).

METHODS

Animals

Morphological studies. Thirty male Sprague-Dawley rats (Bantin and Kingman Ltd, UK), in two separate batches, separated by an interval of several months, were maintained on a vitamin E deficient diet (supplied by ICN Nutritional Biochemicals, USA). It comprised tocopherol stripped corn oil (10%), glucose (66%), salt mix (4%), vitamin-free casein (20%) and full vitamin supplementation (excluding vitamin E). The diet was sterilized by irradiation (1.5 Mrads). The vitamin E content of the diet was determined as being < 1 ng/g. Thirty control rats were administered the same diet but 160 mg/l of Rovomix E-50 SD (Roche Products) was added to their drinking water, calculated to deliver 2.5 mg of α-tocopherol per day to each animal. Food and water were provided ad libitum. All animals for this part of the study were housed at the Royal Free Hospital School of Medicine in an isolation unit (Vickers Medical Gnotoflex Isolator) at a temperature of 21–22° C with an artificial day length of 13 h. Plastic cages were used to avoid the development of pressure neuropathy in the feet which may occur in cages with wire mesh floors. The diet was commenced shortly after weaning. The weights and clinical status of the animals were assessed weekly.

Axonal transport studies. Weanling, specific pathogen free, male Wistar rats (Charles Rivers Ltd, UK) were placed on a vitamin E deficient diet (Machlin/Draper-HLR 814; Dyets, Pennsylvania, USA). A second control group received the same diet to which α-tocopheryl acetate (100 mg/kg) was added. The rats were maintained at the Institute of Child Health on these two diets for 1 yr. Axonal transport studies were performed at 2, 4, 6, 9 and 12 months.

Studies on the effects of dietary polyunsaturated fatty acids (PUFA) and ethoxyquin. These animals were maintained at the Royal Free Hospital School of Medicine under the same conditions as those used for the morphometric studies. Sixty male Sprague-Dawley rats were fed one of the following diets from weaning for 4, 6 or 13 months: (1) vitamin E deficient +0.02% α-tocopherol (controls); (2) vitamin E deficient; (3) vitamin E deficient +15% corn oil (high in PUFA); (4) vitamin E deficient +0.01% ethoxyquin. There were 15 rats in each group. They were assessed at regular intervals using a semiquantitative scale (*see* Table 1).

TABLE 1 CLINICAL ASSESSMENT SCALE

Grade	*Clinical features*
0	No abnormal signs
1	Consistent weight loss (over 4 wks) and/or slight wasting of hind quarters
2	More severe wasting of hind quarters; waddling ataxic gait with tendency for legs to tuck under abdomen; scoliosis
3	Prominent wasting of hind quarters and hind legs; paraparesis; scoliosis
4	Severe paraplegia with wasting; early weakness of forelimbs
5	Tetraparesis and weakness of neck muscles; generalized wasting

Tissue preparation

While under deep anaesthesia with Sagatal the animals were perfused with 50 ml physiological saline through a catheter inserted via the left ventricle into the ascending aorta. This was followed by 350 ml dilute Karnovsky's fixative initially at 37° C and subsequently at 4° C. Samples were taken for morphological study from the optic nerves and tracts, gracile nuclei, dorsal, lateral and ventral columns from the cervical, thoracic and upper lumbar spinal cord, dorsal roots, dorsal root ganglia, proximal (thigh) and distal (lower leg) sural nerves and lumbrical muscles. The specimens were further fixed at 4° C for 4 h in Karnovsky's fixative and postfixed overnight in 1% osmium tetroxide in 0.1 M sodium cacodylate buffer (pH 7.4) containing 5% sucrose, 1.5% potassium ferricyanide and 3% sodium iodate at 4° C. After dehydration through graded concentrations of ethanol, they were embedded in Araldite via propylene oxide. Semithin sections were stained with thionin and acridine orange (Sievers, 1971). Ultrathin sections were stained with uranyl acetate and lead citrate and examined with a JEOL 100CX electron microscope.

Morphometry

Morphometric studies were performed on electron micrographs of transverse sections of the sural nerve at proximal and distal levels. Each complete myelinated fibre visible in low power micrographs (final magnification approximately ×1200) was numbered systematically. Fibres sectioned obliquely, through the paranodal region or through Schmidt-Lanterman incisures, were not included. An initial sample fibre was selected using a table of random numbers and then every tenth subsequent fibre was selected. This ensured that 10% of the population was sampled, that the whole area of the nerve was covered, and that each member of the population had the same chance of being included. To check that the sample size was sufficiently large to be representative of the population, the minimum sample size required to represent a population formed by combining all the samples within an experimental group was calculated, using the following formula (adapted from Hansen *et al.*, 1966):

$$n = \frac{Nz^2 \sigma^2}{Ne^2 + z\sigma^2}$$

where n = minimum sample size, N = population size, z = 1.96 (the acceptable probability that the error can occur, i.e., 95% of the observations will be within the range u ± 1.96 SDs; u = population mean), σ = standard error of the population; e = acceptable error (10%) compared with the mean. The sample size was found to be <10% of the population total for each experimental group.

Fibres which were selected by the sampling procedure were relocated and photographed individually at higher magnification (×5000 or ×6000). The perimeters of myelinated fibres (measured to the outer border of the myelin sheath) and of their axons were measured directly from negatives using a Kontron MOP image analyser. From the measurements obtained, fibre and axon areas were derived. Myelin lamellar counts from which myelin thickness could be derived were obtained from macroenlargements of negatives (final magnification ×500 000) on a TV monitor.

Axonal transport studies

Fast axonal transport of acetylcholinesterase (anterograde and retrograde) was measured in the sciatic nerve using a double ligation/accumulation technique similar to that described by Oikarinen and Kalimo (1984). Rats were sedated with Hypnorm (1 ml/kg) and maintained under deep anaesthesia with a halothane/oxygen/nitrous oxide mixture. The left sciatic nerve was exposed and tightly ligated (using 4–0 braided silk) at the level of the sciatic notch and at the popliteal fossa taking care not to stretch the nerve or to detach it from the surrounding tissue. Three hours after ligation the animal was killed and the whole ligated nerve removed, cleaned and frozen flat. Nerve sections were removed (*see* fig. 1) and homogenized in 400 μl 25 mM phosphate buffer (pH 7.4) containing 0.5% Triton 100X.

Acetylcholinesterase (AChE) was assayed by a modification of the method described by Tuček (1974) which uses the formation of (1-^{14}C)acetate from (1-^{14}C)acetylcholine chloride (305 mCi/mg, Amersham International). Routinely, 50 μl of nerve homogenate were added to 90 μl 20 mM phosphate buffer containing 0.25% Triton 100X and 0.1 mM ethopropazine, 10 μl (1-^{14}C)acetylcholine chloride (5 mCi/ml in 30 mM acetylcholine chloride) and incubated for 1 h at 37° C. After incubation, 250 μl 2 mM eserine salicylate were added and unhydrolysed acetylcholine removed by extracting twice with 500 μl sodium tetraphenylboron in heptanone (15 mg/ml). Radioactivity was counted in 250 μl of the remaining aqueous phase in 10 ml scintillant (Optiphase, Fisons Ltd, UK) on an LKB Wallac Rackbeta liquid scintillation counter using external standardization for quench correction.

The stationary phase (i.e., the amount of AChE normally present in unligated nerve) was determined by the mean DPM/mm present between the ligatures. Anterograde accumulation of AChE was expressed as the percentage increase (relative to the stationary phase) in AChE activity (DPM/mm) in the nerve section proximal to the first ligature. Retrograde accumulation of AChE was expressed as the percentage increase (relative to the stationary phase) in AChE activity (DPM/mm) in the nerve section distal to the first ligature.

RESULTS

Clinical observations

The growth curves for the vitamin E deficient rats varied to some extent between the different batches of animals but did not differ significantly between the deficient and sufficient animals up until 12–16 wks, at which time they began to diverge. From 20–24 wks the sufficient rats were significantly heavier and continued to increase in weight, whereas the deficient rats began to plateau or to decrease in weight.

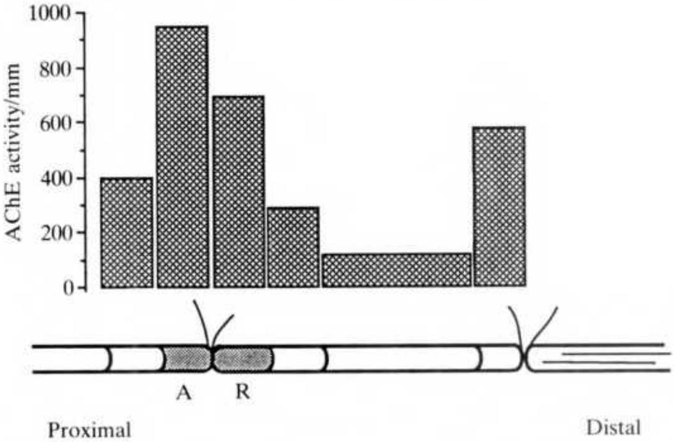

FIG. 1. Determination of anterograde and retrograde axonal transport of acetylcholinesterase in a doubly ligated sciatic nerve. Typical acetylcholinesterase activities in nerve sections after 3 h ligation are shown in the histograms. Anterograde accumulation was expressed as the percentage increase in activity/mm in the section proximal to the first ligature (A) compared with the mean interligature activity/mm. Retrograde accumulation was expressed as the percentage increase in activity in the section distal to the first ligature (R).

After 30–40 wks on the diet, the deficient rats began to exhibit varying degrees of poor coat condition, muscle wasting, hindlimb weakness and a waddling ataxic gait. The hind limbs tended to tuck under the abdomen. Kyphoscoliosis also appeared, probably related to weakness of the paraspinal muscles. These abnormalities became more obvious by 50 wks. Electromyography (concentric needle electrode) of wasted muscles showed increased fibrillary insertion activity and positive sharp waves, and also spontaneous fibrillation, suggesting the presence of denervation.

Qualitative morphological observations

Dorsal columns, gracile and cuneate nuclei, and visual pathways. It was evident that age, the level of the spinal cord and the vitamin E status all affected the severity of the pathology present. Although similar pathological changes were found both in the vitamin E deficient and control animals, they were much more frequent in the former. The abnormalities were more prominent in the gracile fasciculi. The dorsal columns in the thoracic region were almost completely normal both in the deficient and control animals at 4 months and in the lumbar region at 8 months. Wallerian-type axonal degeneration and axonal dystrophy were occasionally encountered in the gracile nuclei after 4 months and in the cervical and thoracic posterior columns at 8 months in both groups. Sections obtained from the gracile and cuneate nuclei at 8 and 12 months and in the cervical, thoracic and lumbar dorsal columns revealed a greater degree of pathology in the deficient animals, which was particularly evident at 12 months.

Both myelinated and unmyelinated dystrophic axons were observed, including large axonal expansions lacking a myelin ensheathment (fig. 2) or surrounded by an attenuated layer of myelin. Retraction of myelin loops was seen at nodes of Ranvier.

No morphological changes were detected in the optic nerves or tracts.

The perikarya of the neurons of the gracile and cuneate nuclei appeared normal, apart from containing increased numbers of lipofuscin granules, especially in the 12 month deficient animals.

Ultrastructure of the dystrophic axons. Fig. 2, a transverse section through the rostral dorsal columns just caudal to the medullary relay nuclei from a deficient animal, shows a number of enlarged dystrophic axons. While many axons are severely dystrophic, others appear normal. Longitudinal sections revealed that the swelling was not continuous along the length of an affected axon but occurred abruptly as the result of material accumulating in the axon (fig. 3A, B).

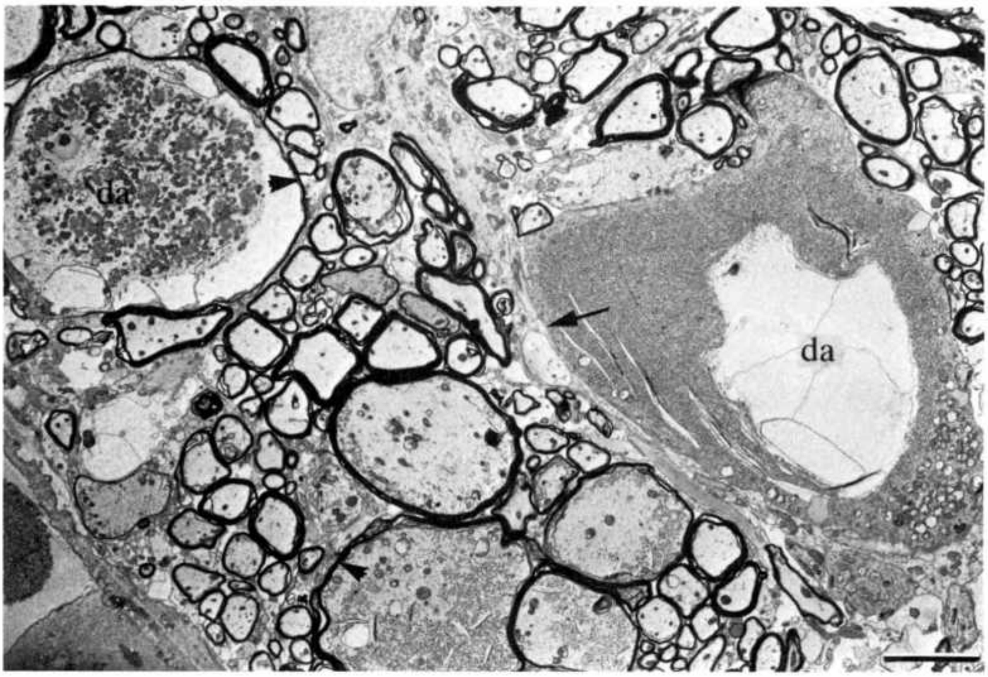

FIG. 2. Electron micrograph from rostral dorsal columns of a rat deficient in vitamin E for 12 months showing enlarged dystrophic axons (da) filled with a variety of accumulated materials and either lacking a myelin sheath (arrow) or surrounded by an attenuated sheath (arrowheads). Bar = 5 μm.

Tubulovesicular structures (tvs) were frequently seen in dystrophic axons and were sometimes the only indication of an axonal disturbance. In mild instances, tvs closely resembled smooth endoplasmic reticulum and consisted of branched tubules of uneven outline measuring 25 nm in diameter. The tvs either accumulated in patches (fig. 4A) or formed a halo around a central core of neurofilaments (fig. 5) or themselves formed a central core (fig. 4B). At times, they occupied almost all the axoplasmic space. They became packed into dense clusters of varying size in which the component branching tubules, although less readily seen, were still discernible (fig. 4B, 6A). In all the large collections of tvs, arrays of tightly packed membranes were seen, which were apparently

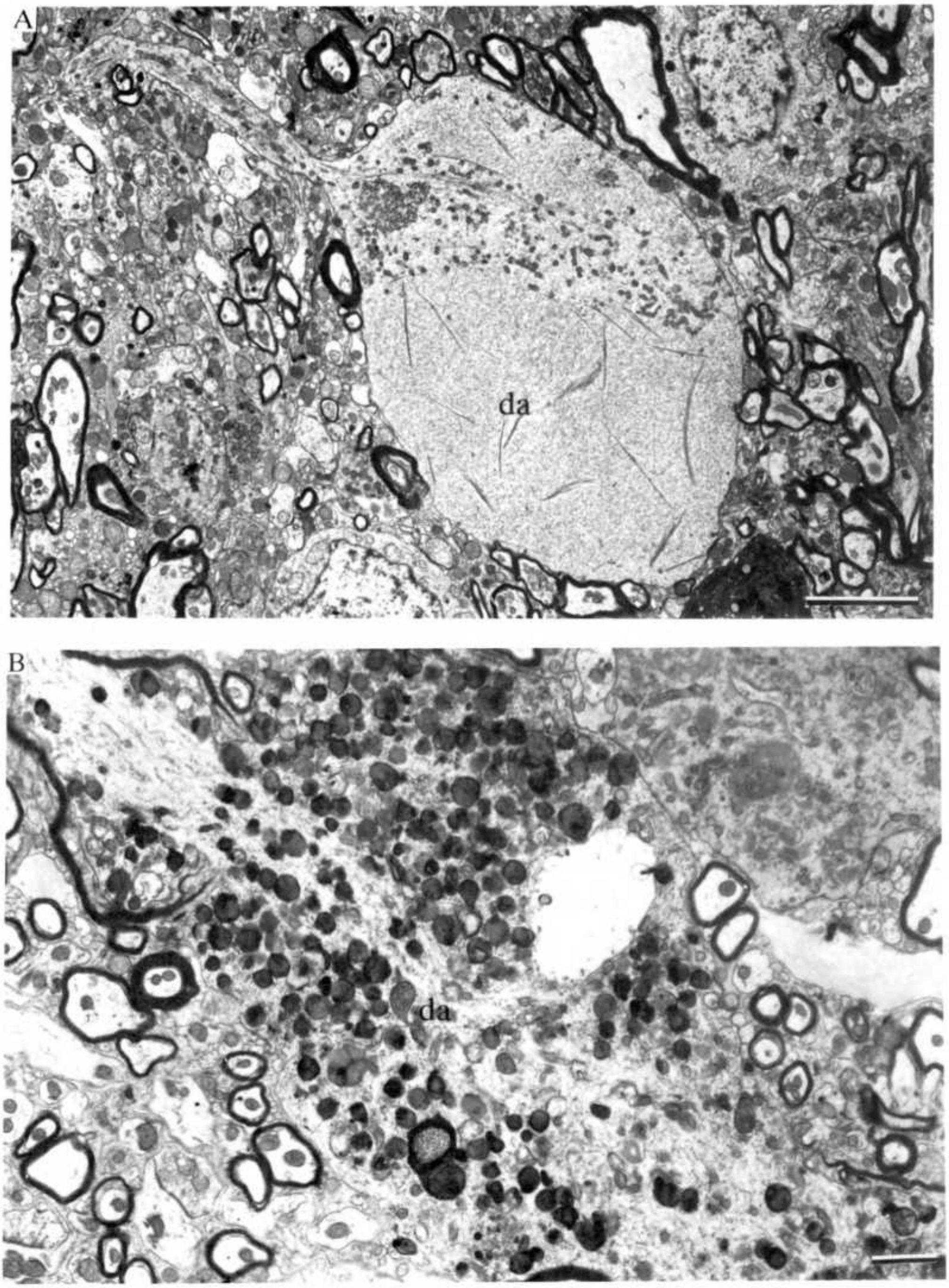

FIG. 3. A, electron micrograph of a longitudinally sectioned axon from the rostral gracile nucleus of a 12 month control animal showing a focal dystrophic expansion (da). Bar = 5 μm. B, electron micrograph of a longitudinal section through a node of Ranvier in the gracile nucleus from a 12 month control animal showing an expanded dystrophic axon (da) filled with a variety of organelles. Bar = 1 μm.

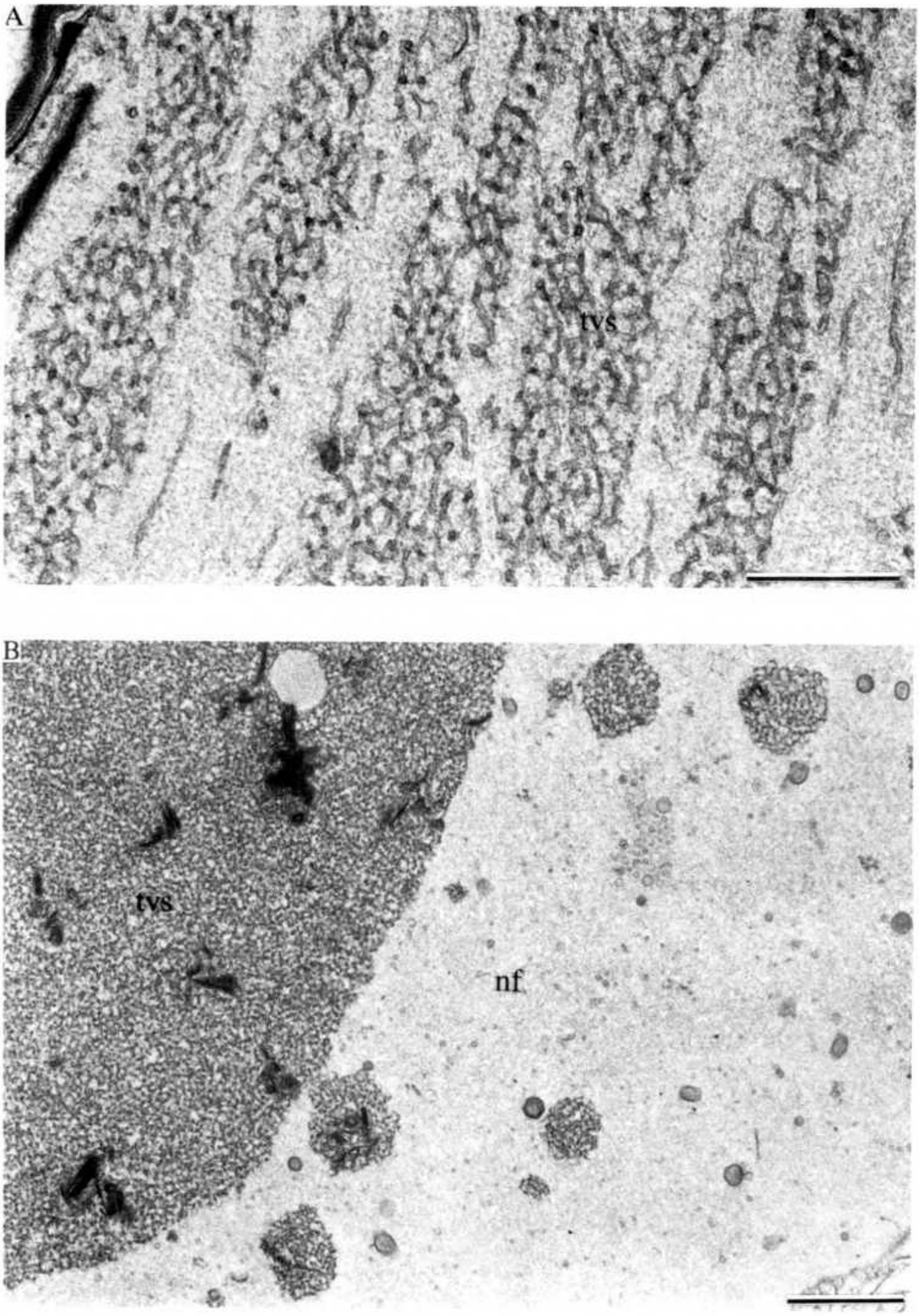

FIG. 4. A, electron micrograph of a longitudinal section through a dystrophic axon from the gracile fasciculus of an animal deficient in vitamin E for 12 months showing accumulations of tubulovesicular structures (tvs). Bar = 0.5 μm. B, electron micrograph of transverse section through a dystrophic axon from the gracile nucleus of a 12 month control animal showing a central accumulation of tubulovesicular structures (tvs) surrounded by neurofilaments (nf). Bar = 1 μm.

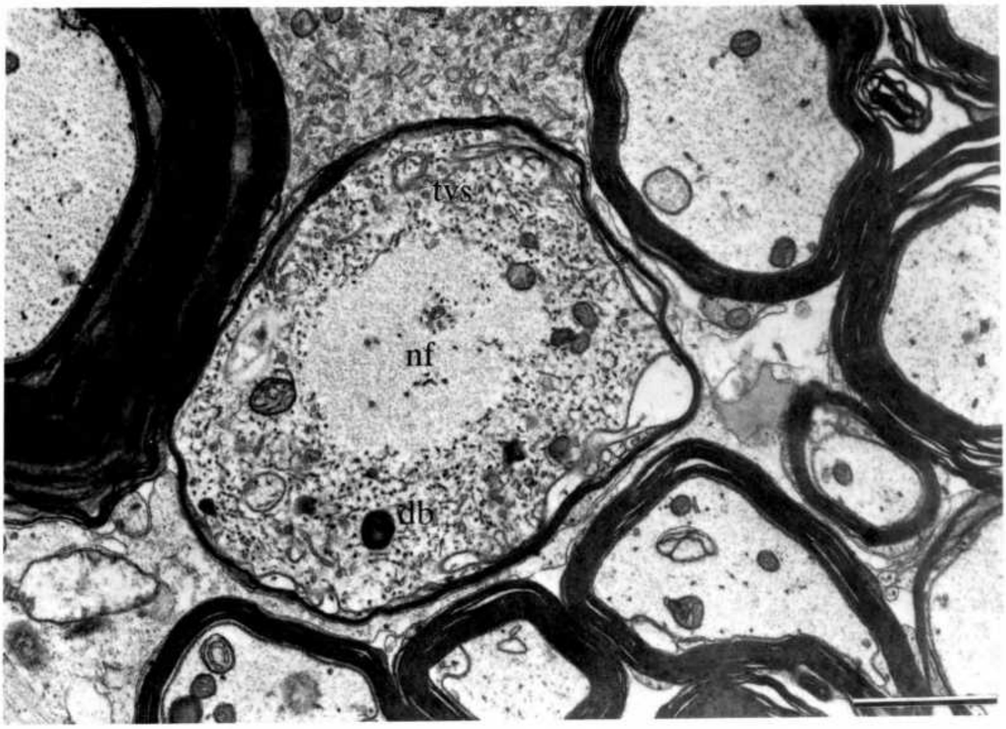

FIG. 5. Electron micrograph of a transverse section through a moderately dystrophic axon from the cervical gracile fasciculus of an animal deficient in vitamin E for 12 months showing a central core of neurofilaments (nf) surrounded by a halo of accumulated tubulovesicular structures (tvs) and other material, including a dense lamellar body (db). Bar = 1 μm.

continuous with tvs (fig. 6A, B). Both the tubules and lamellae were sometimes dilated, forming vacuoles that occasionally reached 25 μm in diameter and which contained granular material (fig. 6B).

A very prominent feature of a small number of dystrophic axon terminals in the gracile nuclei was the presence of extensive arrays of layered looped membranes (fig. 7A). Individual membranes were 40–80 nm from their neighbours and were observed to be continuous with tightly packed clusters of tvs (fig. 7B).

Accumulations of neurofilaments were also frequently observed but were not an essential prerequisite for axonal swelling. They were sometimes observed in patches amongst aggregates of tvs, often not aligned in the long axis of the axon. As mentioned above, they sometimes formed a central core to the axon and were surrounded by accumulated organelles (fig. 5) or surrounded a central core of tvs (fig. 4B). Changes in microtubules were less notable, but occasional axons showed an abnormally high density of these structures.

Focal accumulations of mitochondria were very common (figs 2, 3B). They were frequently associated with a varied selection of organelles and other material. Many of the mitochondria in such accumulations appeared normal; others were distorted, being enlarged and vacuolated with displaced cristae. Some contained fine granular material.

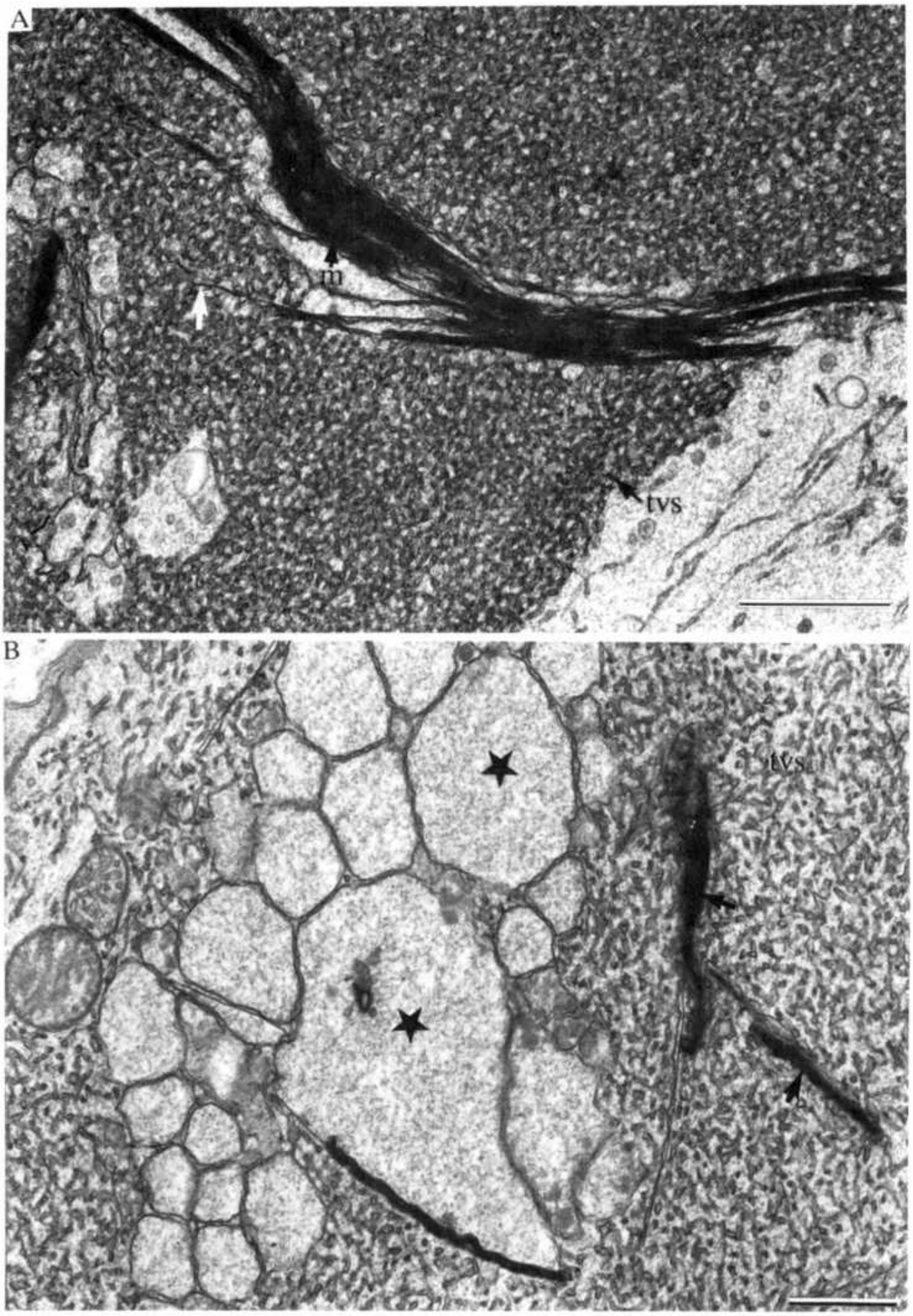

FIG. 6. A, electron micrograph through part of a dystrophic axon from the gracile nucleus of an animal deficient in vitamin E for 12 months showing accumulated tubulovesicular structures (tvs) and tightly packed membranous arrays (m). There is apparent continuity between the branched tubules and the lamellae of the membranes (arrows). Bar = 0.5 μm. B, electron micrograph through part of a dystrophic axon from the gracile nucleus of an animal deficient in vitamin E for 12 months showing dilated membrane-bound spaces (asterisks) apparently derived from tubulovesicular structures (tvs) and their associated parallel arrays of membranes (arrows). Bar = 0.5 μm.

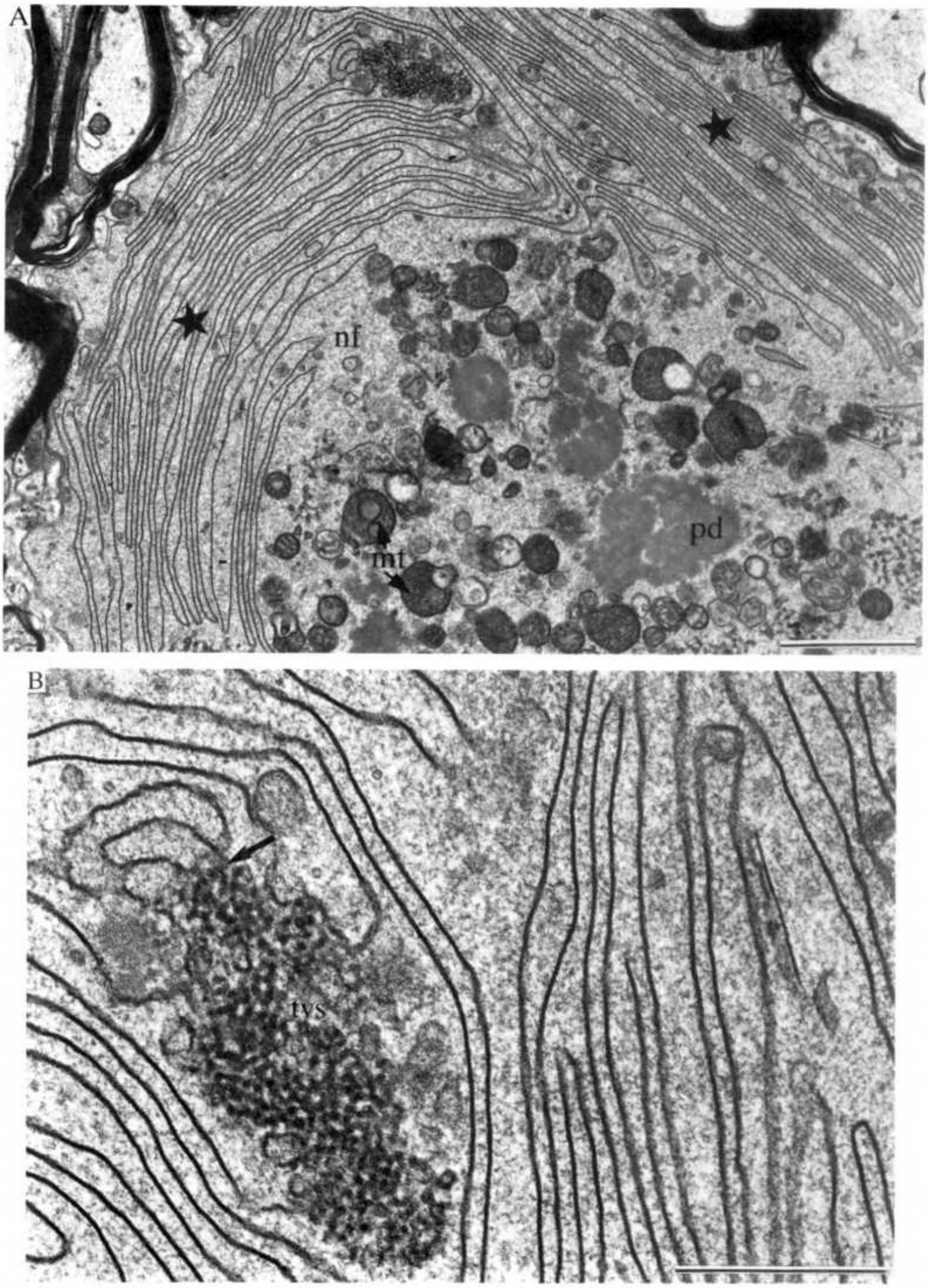

FIG. 7. A, electron micrograph through a dystrophic axon terminal from the gracile nucleus of a 12 month control animal showing prominent layered loops of membranes (asterisks) surrounding an accumulation of neurofilaments (nf), patchy dense granular material (pd), mitochondria (mt) and other structures. Bar = 1 μm. B, detail from A showing continuity of the layered loops of membranes and tubulovesicular structures (tvs) at arrow. Bar = 0.5 μm.

Dense lamellar bodies (fig. 5) were usually of similar size (0.2 μm diameter) and shape to mitochondria and consisted of closely packed concentrically arranged lamellae surrounding a pale centre.

Vesicles and multivesicular bodies (MVB) were also commonly found in accumulations of organelles and other material along with mitochondria, dense lamellar bodies and secondary lysosomes. The MVB displayed a variety of sizes and shapes. Small (39–60 nm) round vesicles, surrounded by a single membrane, were commonly encountered. They resembled synaptic vesicles but were found in dystrophic axons remote from presynaptic membranes.

Glycogenosomes were less common than mitochondria, dense lamellar bodies, vesicles and MVB. They were observed in association with accumulated organelles in dystrophic axons and also in isolation in the axoplasm of apparently unaffected fibres. They attained diameters of up to 2 μm and were filled with large numbers of small (15 nm) osmiophilic glycogen granules. An irregular membrane was usually but not always seen to envelop the glycogenosomes.

Patches of dense fine granular material were commonly seen in association with accumulations of organelles. Small patches, 0.2 μm in diameter, tended to coalesce to form larger patches. This material was not membrane bound and had an irregular indistinct outline. It resembled material also seen within tvs and mitochondria. On rare occasions, large numbers of randomly orientated tubular rings, ~75 nm in diameter, could be discerned within larger areas of patchy dense material (fig. 8).

Dorsal roots and dorsal root ganglia. Dystrophic axons were not observed in the dorsal roots or dorsal root ganglia of either control or deficient animals at any of the ages examined. Dorsal root ganglion cells appeared normal apart from a tendency for increased numbers of cytoplasmic lipofuscin granules with ageing both in the control and deficient animals, although this was most apparent in the 12 month deficient animals.

Distal limb nerves. Intramuscular nerves in the lumbrical muscles and nerve fibres within the small foot muscles were examined. Only myelinated fibres were assessed in the spindles to eliminate possible confusion with nonpathological accumulation of organelles in terminals. The changes were more prominent in the muscle spindles and although dystrophic axonal changes were observed, they were not as striking as in the rostral spinal cord. At the 4 month stage, abnormalities were not detected, either in the deficient or control animals. By 8 and, more particularly, 12 months, a number of fibres showed a variety of inclusions, especially in the deficient animals (fig. 9). These comprised smooth endoplasmic reticulum, vesicles and vacuoles, mitochondria (some of which had distorted cristae), dense lamellar bodies, MVB, patchy dense material and glycogenosomes. Such accumulations often filled the axon, completely disorganizing the normal cytoskeleton. Swelling of axons to the extent seen in the spinal cord was not observed and large dense accumulations of tvs or neurofilaments were not encountered. Glycogenosomes tended to be more numerous.

Sural nerve morphometry

Observations were made on the proximal and distal sural nerve in deficient and control animals at 4, 8 and 12 months for the first batch of animals (group 1) and at 8 and 12 months in the second batch (group 2). A comparison between the deficient and control animals is given in Tables 2 and 3. The frequency distribution for axon perimeters and

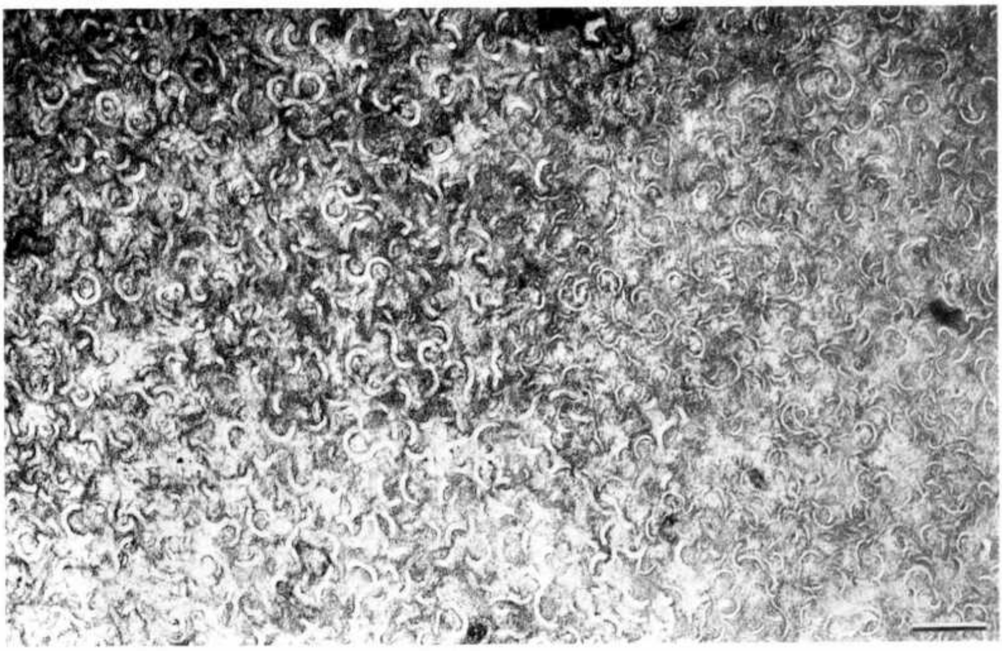

FIG. 8. Electron micrograph through part of a dystrophic axon from the gracile nucleus of an animal deficient in vitamin E for 12 months showing numerous curvilinear tubular structures with a diameter of 75 nm. Bar = 0.2 μm.

areas was unimodal at all stages whereas that for the number of myelin lamellae was bimodal (fig. 10), as is true for the tibial nerve in the rat (Fraher *et al.*, 1990). The means and distributions were compared by analysis of variance.

There were no significant differences between the deficient and control animals for any of the variables investigated (total fibre area or perimeter, i.e., measured to the outer circumference of the myelin sheath, axonal area or perimeter, numbers of myelin lamellae) at 4 or 8 months, either in the proximal or distal sural nerve. At 12 months, for the proximal sural nerve, mean axonal perimeter for myelinated fibres was significantly greater in the deficient animals (fig. 10A, B). There was also a tendency for axonal area and myelinated fibre area and perimeter to be increased in the deficient animals. Conversely, myelin sheath thickness, represented by the lamellar counts, was significantly greater in the control animals (fig. 10C, D). In the distal sural nerve, the mean values for axonal areas and perimeters were both significantly greater at the 12 month stage in the deficient animals (fig. 10E, F). There was a tendency for the myelin lamellar count to be greater in the control animals, but this did not reach statistical significance.

Axonal transport studies

The AChE in the stationary phase (i.e., the amount of AChE normally present along the nerve) was found to be the same in the nerves of both the vitamin E deficient and control rats during all stages of the study. Changes in accumulation were therefore assumed to be due to the rate of transport rather than the amount transported.

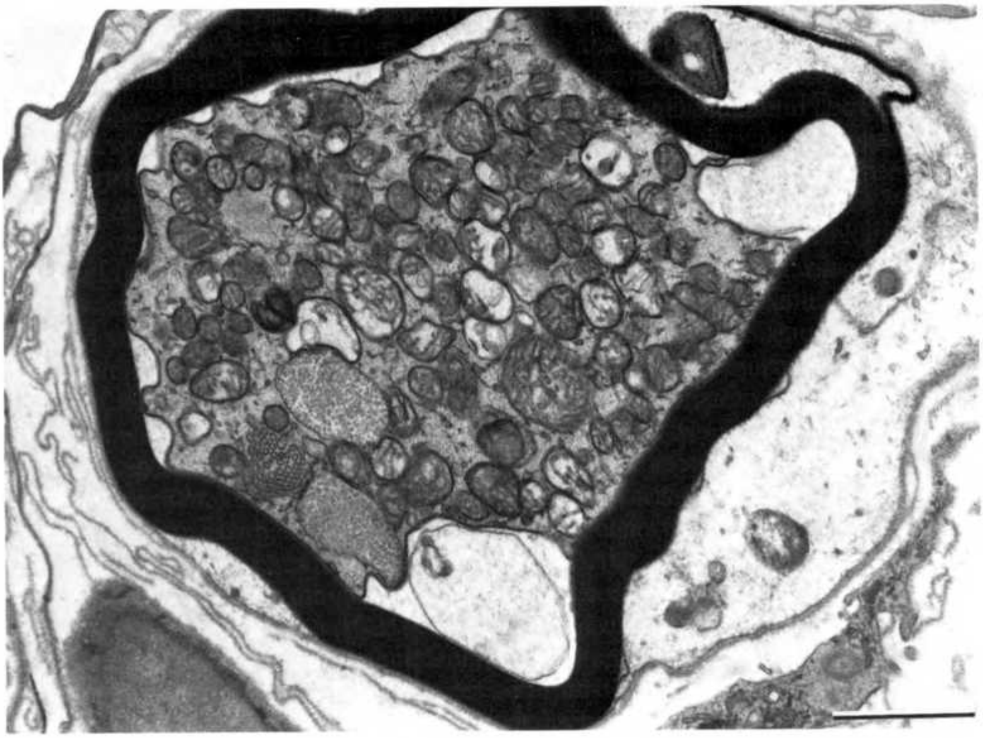

FIG. 9. Electron micrograph of transverse section through a myelinated axon within a muscle spindle from a lumbrical muscle of an animal deficient in vitamin E for 12 months. The axon is filled with a variety of organelles. Bar = 1 μm.

TABLE 2. MORPHOMETRIC RESULTS FOR PROXIMAL SURAL NERVE

	Duration of dietary regimen (months)	*Control groups*		*Deficient groups*		*ANOVA F value*	*P*
		1	*2*	*1*	*2*		
No. of animals		14*	16**	14*	16**		
Fibre area (μm^2)	4	18.32	–	20.75	–	4.84	n.s.
	8	25.97	26.76	28.13	28.19	2.56	n.s.
	12	27.44	25.16	28.40	28.48	3.58	n.s.
Axon area (μm^2)	4	7.26	–	8.12	–	3.03	n.s.
	8	11.09	9.23	11.49	11.29	2.31	n.s.
	12	10.96	10.81	11.66	12.57	4.47	n.s.
Fibre perimeter (μm)	4	17.89	–	19.04	–	4.53	n.s.
	8	20.90	21.73	21.83	22.19	4.40	n.s.
	12	21.76	20.01	22.11	21.79	6.15	n.s.
Axon perimeter (μm)	4	12.67	–	13.45	–	4.12	n.s.
	8	15.15	14.98	15.46	15.93	3.91	n.s.
	12	15.31	14.37	15.94	15.88	10.42	< 0.01
Lamellar count	4	50.50	–	54.52	–	4.92	n.s.
	8	54.23	57.99	59.71	55.66	1.19	n.s.
	12	60.18	56.61	54.81	54.39	7.14	< 0.01

* 4 animals examined at first time interval, 5 at second and third. ** 8 animals examined at both time intervals.

TABLE 3 MORPHOMETRIC RESULTS FOR DISTAL SURAL NERVE

	Duration of dietary regimen (months)	*Control groups*		*Deficient groups*			
		1	*2*	*1*	*2*	*ANOVA F value*	*P*
No. of animals		14*	16**	14*	16**		
Fibre area (μm^2)	4	20.06	–	19.48	–	0.26	n.s.
	8	24 81	22 66	24 41	26 21	2.81	n.s.
	12	23.74	21.95	25.00	25.27	5.36	n.s.
Axon area (μm^2)	4	7.96	–	7.72	–	0.23	n.s.
	8	10 52	8.29	9.97	10.46	3.11	n.s.
	12	9.50	8.86	10 44	10.91	10.42	< 0.01
Fibre perimeter (μm)	4	18.43	–	18.69	–	0.21	n.s
	8	20.27	20 39	20.21	21 66	3.17	n s
	12	20.52	19.08	20.79	20.44	2.57	n.s
Axon perimeter (μm)	4	12.78	–	13.05	–	0.45	n.s.
	8	14.51	14.49	14.33	15.05	2.57	n.s
	12	14.40	13.55	14.90	14.72	8.88	< 0.01
Lamellar count	4	55 00	–	54.69	–	0.03	n.s
	8	53 90	57.40	57.62	58.92	4.05	n.s
	12	59.00	53.33	57.05	53.28	0.26	n.s.

* 4 animals examined at first time interval, 5 at second and third. ** 8 animals examined at both time intervals.

Accumulation of AChE due to anterograde transport in the deficient and control rats is shown in fig. 11A. There were no significant differences until 52 wks when the deficient rats exhibited a 25% reduction in AChE accumulation ($P < 0.02$). Retrograde accumulation is presented in fig. 11B. There were again no differences until 52 wks, when the deficient rats exhibited a 20% reduction in AChE accumulation ($P < 0.05$).

Effects of dietary polyunsaturated fatty acids (PUFA) and ethoxyquin

Control rats remained healthy throughout. Vitamin E deficient rats were significantly less heavy than controls by 6 months. The first clinical signs appeared at 10 months. At 13 months all 5 rats maintained to this stage had developed the signs of deficiency described earlier. Vitamin E deficient rats administered PUFA developed clinical signs much earlier than deficient animals not given PUFA (2 months compared with 10 months). By 4 months the signs were severe, and by 6 months this group had to be killed. At that stage they showed severe wasting and weakness of the hindlimb, forelimb, and neck muscles. Scoliosis was obvious, and weight loss dramatic. Of the deficient rats to which ethoxyquin was administered, 2 out of 5 showed clinical signs by 13 months when the experiment was terminated. The mean clinical scores for the animals in the four groups are shown in fig. 12.

DISCUSSION

As stated in the Introduction, the morphological changes in the nervous system associated with vitamin E deficiency indicate a length-related central-peripheral distal axonopathy (Spencer and Schaumburg, 1976) with greater involvement of the centrally-directed axons of the primary sensory neurons, particularly those in the gracile fasciculi.

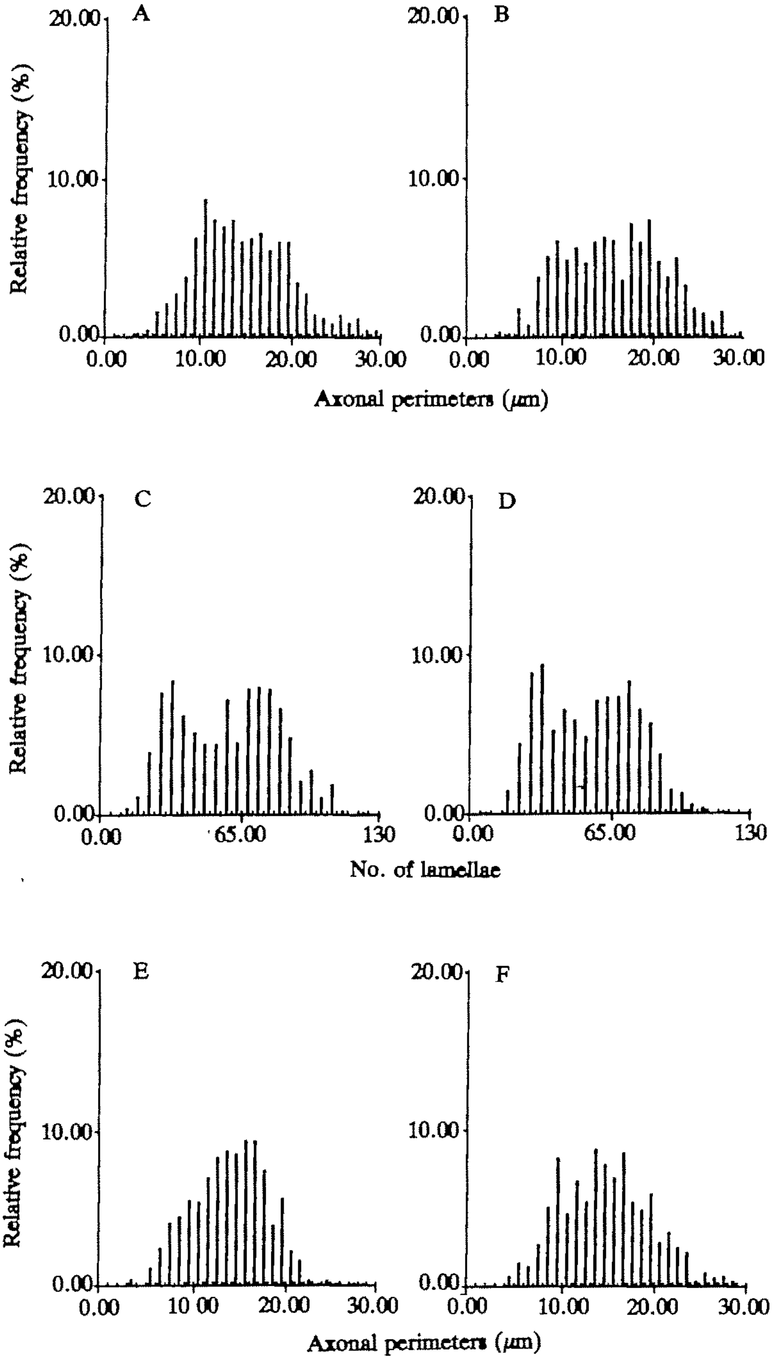

FIG. 10. Size-frequency distributions for myelinated fibres in the sural nerve, for axon perimeters (A, B) and numbers of myelin lamellae (C, D) in the proximal sural nerve, and axonal perimeters in the distal sural nerve (E, F). A, C and E are from 12 month control rats and B, D and F from animals deficient in vitamin E for 12 months.

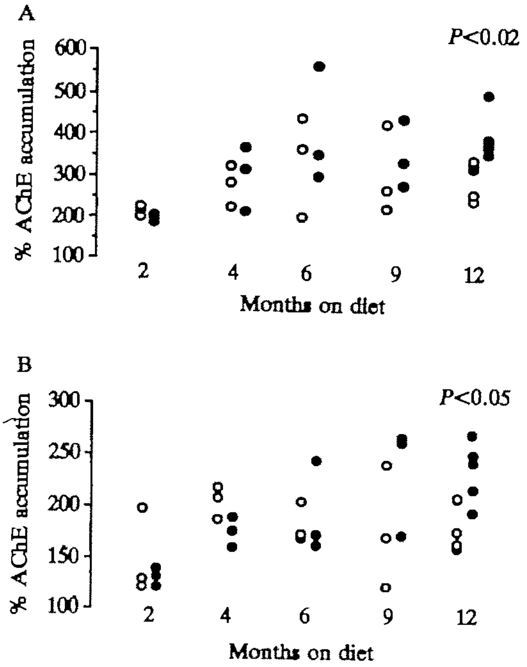

Fig 11. Longitudinal anterograde (A) and retrograde (B) axonal transport of acetylcholinesterase in vitamin E deficient (open circles) and age-matched controls (filled circles).

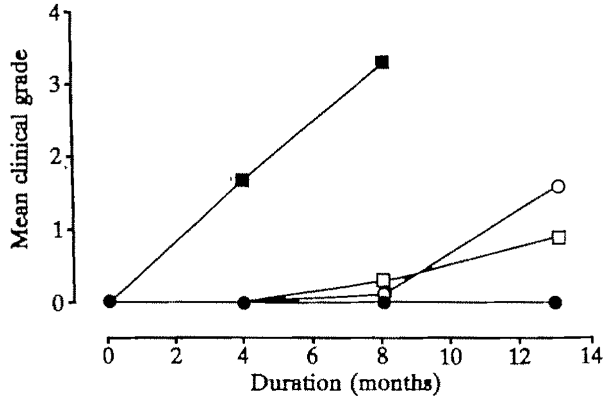

Fig. 12. Changes in clinical scores for controls (closed circles, n = 15), and rats deficient in vitamin E (open circles, n = 16), deficient+ethoxyquin (open squares, n = 16) and deficient+polyunsaturated fatty acid (PUFA) (filled squares, n = 16).

Thus the changes were maximal in the rostral parts of the dorsal columns and greater there than in the distal peripheral nerves. The long descending tracts were not examined in the present study. The most striking feature of the pathological changes was the occurrence of axonal dystrophy in the rostral parts of the dorsal columns with the presence of axonal swellings containing accumulations of a variety of organelles. These changes are not specific and have been described, for example, in normal ageing (Seitelberger, 1971; Fujisawa and Shiraki, 1978; Fujisawa, 1988), as was also observed in present material. The dystrophic axons contained a variety of organelles, including normal mitochondria, tubular and vesicular elements and neurofilaments, together with abundant degenerate mitochondria, dense lamellar bodies, multivesicular bodies and secondary lysosomes. All these structures are known to be carried in the retrograde axonal transport system (Tsukita and Ishikawa, 1980). The appearances are therefore strongly suggestive of an abnormality of 'turnaround' whereby materials that have descended the axon in the anterograde transport system are packaged into lysosomes for return to the perikaryon in the retrograde transport system. This is thought to be involved in certain toxic neuropathies including those due to zinc pyridinethione (Sahenk and Mendell, 1980), acrylamide (Sahenk and Mendell, 1981) and *p*-bromophenylacetylurea (BPAU), in which the neurological changes are similar (Jakobsen and Brimijoin, 1981). There is a striking accumulation of tubulomembranous material in distal and preterminal axons in these neuropathies (Blakemore and Cavanagh, 1969; Ohnishi and Ikeda, 1980). The accumulation of smooth membranous profiles has been observed in dystrophic axons in peripheral nerve in human vitamin E deficiency (Brin *et al.*, 1985).

The present study has revealed a similar striking accumulation of membranovesicular structures in distal axons in experimental vitamin E deficiency. We have also found a reduction in the velocity of fast anterograde and retrograde transport AChE but no indication of a reduction in the total quantity transported. A similar finding has been obtained related to ageing in rats (McMartin and O'Connor, 1979). Slow axonal transport was not examined but, as there was no evidence of axonal atrophy, or of giant axonal neuropathy, it is unlikely that it is affected. The cytoskeletal components of axoplasm, namely neurofilaments and microtubules, are carried by the slow anterograde system and, if delivery is impaired, focal accumulations of neurofilaments ('giant axonal neuropathy') develop with axonal atrophy distal to the accumulation (Hoffman *et al.*, 1984).

Axonal transport studies in experimental vitamin E deficiency have so far been very limited. Roessmann and Friede (1967), using intraperitoneal ^{3}H-leucine and autoradiography, concluded that the dystrophic swollen axons were not due to excessive local synthesis of protein but to a disturbance in the equilibrium between the rate of anterogradely transported proteins and the local rate of protein degradation. Wood and Boegman (1975) reported an increase by ×2.4 (to 900 mm/day) in the rate of anterogradely transported proteins in motor axons of vitamin E deficient rats following the injection of ^{3}H-leucine into lower lumbar ventral horns. They concluded that the increase in the rate of transport was due to an increased demand on the nerve for trophic substances from degenerate muscles. Such an increase in the rate of axonal transport has not, however, previously been reported in any other condition.

The present morphometric findings in the sural nerve call for comment. A slight increase in axonal diameter was found, both at the proximal and distal levels. Such an alteration has not previously been detected in experimental neuropathies, but was found independently in both batches of animals subjected to morphometric study. It suggests that the accumulation of materials is not confined to the axonal terminations but is expressed to some extent at more proximal levels despite the lack of obvious associated ultrastructural changes.

Vitamin E is the only well recognized lipid-soluble chain-breaking antioxidant in vivo (Burton *et al.*, 1983*a, b*). It is assumed that, as a result of its antioxidant properties, it is able to terminate lipid peroxidation in membrane in neural tissues and thereby prevent the characteristic neurological abnormalities associated with vitamin E deficiency. Some evidence for this hypothesis was provided by Nelson (1987) who showed that the characteristic neuropathology of vitamin E deficiency in the rat could be prevented by the addition of the synthetic antioxidants ethoxyquin and promethazine. This study provides further evidence that vitamin E is acting as an antioxidant in vivo. First, we have confirmed that ethoxyquin can reduce the development of the neurological syndrome and, secondly, have shown that the addition of excess peroxidizable substrate in the form of PUFA markedly accelerates the rate of development of the neurological syndrome in vitamin E deficient rats. At present it is uncertain as to how much of the neurological syndrome exhibited by vitamin E deficient animals is related to peripheral nerve and CNS changes and how much might be caused by myopathy. The EMG studies that we undertook showed evidence of denervation. Extensive morphological observations on muscle were not undertaken, but those on the lumbrical muscles showed grouped fibre atrophy and small angular fibres consistent with denervation.

The question raised in the Introduction to this paper was why vitamin E deficiency should result in a distal axonopathy. The chemical composition of biological membranes varies considerably. Molenaar *et al.* (1972) categorized cell membranes according to the degree of unsaturation of their component lipids. Plasma, lysosomal and Golgi membranes contain high proportions of saturated and monounsaturated fatty acids, cholesterol and sphingomyelin. The membranes of mitochondria and smooth endoplasmic reticulum (SER) contain a high proportion of polyunsaturated fatty acyl chains and less sphingomyelin. They may well therefore be more susceptible to damage in vitamin E deficiency. In addition, there is increased production of oxygen-derived free radicals in mitochondria as a by-product of oxidative phosphorylation.

On the basis of this background, a hypothesis can be proposed for the development of the distal axonopathy of vitamin E deficiency. If there is a disturbance of the axonal mitochondria related to their increased vulnerability to damage by free radicals, this might be expected to lead to abnormalities in fast anterograde and retrograde transport as both are energy dependent. Presumably the turnaround mechanism in the preterminal regions of the axons would also be affected. The accumulation of organelles, as suggested by Brimijoin (1984), could then 'plug off' the terminal axons so that they become isolated from the cell body and ultimately degenerate. This process could then spread centripetally in a 'dying-back' manner. Why the centrally directed axons of the primary sensory neurons should be affected to a greater extent is uncertain and requires further study. The distal accumulation of membranovesicular profiles derived from the SER is an obtrusive finding and it is noteworthy that, in addition to mitochondria, the SER would be expected to be vulnerable to free radical damage. The composition of the membranes of the axoplasmic vesicles that are the vector of fast axoplasmic transport is not known.

ACKNOWLEDGEMENTS

Financial support from the Friedreich's Ataxia Group, the Leverhulme Trust and Hoffmann-La Roche Ltd is gratefully acknowledged. The electron microscope was provided by the Medical Research Council and the image analysis equipment by grants from Ciba-Geigy Ltd, Basel, and the Central Research Fund of London University. We are indebted to Jane Workman and John Muddle for technical assistance, Kieran Price for secretarial help and Professors A. T. Diplock and J. A. Lucy for helpful advice.

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(Received March 23, 1990. Revised June 5, 1990. Accepted June 12, 1990)

Brain (1991), **114**, 937–950

AGE OF ONSET IN SIBLINGS CONCORDANT FOR MULTIPLE SCLEROSIS

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SUMMARY

We have evaluated genetic and environmental influences in multiple sclerosis (MS) by comparing age of onset in 99 sibling pairs concordant for the disease. We used three methods of analysis: (1) comparison of mean differences in age of onset and year of onset, (2) linear regression of differences in age or year of onset vs difference in ages, and (3) intraclass correlation of age of onset which is also used for monozygotic twins concordant for MS. Comparison of the mean differences in age of onset or year of onset is found to be inappropriate and potentially misleading. No significant results were found in linear regression of the age of onset or year of onset vs differences in ages, although a trend towards onset at the same age is present. However, nontwin siblings show a significant intraclass correlation for age of onset ($P < 0.01$) as is seen in genetic disorders. A stronger intraclass correlation in age of onset in concordant monozygotic twins vs concordant sibling pairs further suggests that age of onset is partly under genetic control, assuming common exposure to an environmental agent. The results give little support for common exposure to an environmental trigger in concordant MS sibling pairs. They are consistent with a mixture of random independent exposures and common exposures leading to the development of the disease, with the former predominating.

INTRODUCTION

Both genetic and environmental factors appear to interact in the development of multiple sclerosis (MS), although the mechanism of this interaction remains uncertain. Support for important environmental determinants of MS include migration data (Kurtzke, 1983); geographic tiering, both in the United States (Kurtzke *et al.*, 1979), and elsewhere (Kurtzke, 1980; Hammond *et al.*, 1988); apparent 'epidemics' in the Faroe Islands (Kurtzke and Hyllested, 1979), and perhaps Iceland (Kurtzke *et al.*, 1982); and the observation of a concordance rate under 40% for monozygotic twins, even with age of onset correction and examination of discordant twins by magnetic resonance scanning (Ebers, 1986).

In the present study, we examine age and year of onset in a population of nontwin siblings concordant for MS to evaluate three hypotheses: (1) common exposure, (2) random exposure, (3) genetic determination. *Common exposure.* If common exposure to a triggering agent were followed by a similar incubation period for each member of an affected sibling pair, would such pairs be expected to develop MS in approximately the same calendar year. *Random exposure.* If development of MS in siblings were due to random independent exposure rather than common exposure to a triggering agent, the age of onset distribution would be expected to be the same in the older sibling as

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for the younger sibling (and the same as the general population of MS patients). The mean difference in age of onset would approximate to zero. *Pure genetic.* If MS susceptibility and the timing of the disease onset were purely under genetic control, it might be expected that onset would tend to be at approximately the same age among sibling pairs.

A series of calculations was developed (Schapira *et al.*, 1963) which, although arbitrary, attempted to evaluate the age of onset in affected sibling pairs as a way of demonstrating common exposure to an infectious agent as the cause of MS. In this paper, we discuss, modify and extend the original approach in a larger population-based data set.

METHODS

Case ascertainment

Nontwin sibling pairs concordant for MS were ascertained through the MS Clinics in Vancouver, British Columbia (n = 1200) and London, Ontario (n = 1400). The latter group included 6 sibships (4 from the University of Calgary and 2 from the University of Ottawa) which were incorporated into the London data as part of another study (Ebers *et al.*, 1982). Otherwise, these pairs represent consecutive ones identified through systematic review of the family histories of all clinic patients. In sibships with more than 2 affected siblings, 1 concordant pair was randomly selected for inclusion in the study. Concordant monozygotic twins were ascertained as part of a nationwide population-based study (Ebers *et al.*, 1986).

Diagnosis

The diagnosis of MS was made by clinic neurologists with special experience in MS and its differential diagnosis. All index cases were diagnosed as having clinically definite (Schumacher *et al.*, 1965) or laboratory supported probable MS (Poser *et al.*, 1984). Whenever possible, reportedly affected siblings of index cases were examined by an MS clinic neurologist. This was possible in over 95% of those siblings still alive in the London series. If this was not possible, medical or autopsy records were obtained and reviewed. Affected siblings of index cases were diagnosed as having clinically definite MS, laboratory supported probable MS, possible MS (Rose *et al.*, 1976), or optic neuritis (Ebers *et al.*, 1981).

Age of onset

For the great majority of affected siblings, age of onset was determined before the initiation of this study. When it had to be determined subsequently, age of onset was assigned without knowledge of the age of onset in the other affected sibling. The age of onset was defined as the earliest symptom(s) believed by the neurologist to represent a manifestation of the disease. Year of onset was the calendar year in which this earliest symptom(s) occurred. For each patient, the earliest medical records available were reviewed to corroborate the reported age and year of onset to minimize the problems of retrospective recall for early symptoms. Discrepancies were resolved by the clinic neurologist after clarification with the patient often with the help of a spouse or other relative.

Data analyses

Fig. 1 illustrates how the calculations of differences in age, age of onset, and year of onset among nontwin sibling pairs were derived. Fig. 2 summarizes the methods of data analysis in conjunction with the expected results from the 3 hypotheses—'common exposure', 'random exposure', and 'pure genetic'. It is clear that the use of mean differences will not distinguish between the 'common exposure' and 'pure genetic' hypotheses. Intraclass correlation will allow distinction between the 'common exposure' and 'random exposure' hypotheses. Regression analysis has the potential to distinguish among the three hypotheses.

Mean differences in age and year of onset. The differences between siblings in year of onset was determined by arbitrarily subtracting the year of onset for the oldest sibling from that for the younger of the pair. The difference in year of onset would be greater than zero if the older sibling were the first to develop symptoms. The difference in age of onset was obtained by subtracting the age of onset for the younger sibling from that for the older sibling. The difference in age of onset would be greater than zero if the

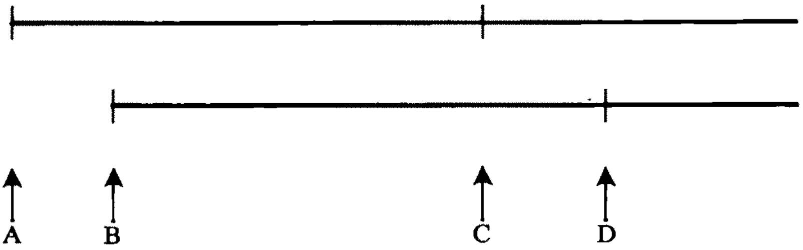

FIG. 1. Diagrammatic representation of ages of onset in concordant sibling pairs A = year of birth of older sibling; B = year of birth of younger sibling, C = year of onset of older sibling, D = year of onset of younger sibling. (D-C = difference in year at onset between siblings; AC-BD = difference between siblings in age at onset, AC-BD/N = mean difference in age at onset; D-C/N = mean difference in year at onset)

older sibling had developed MS at an older age compared with the younger sibling. A two-tailed paired t test (Zar, 1974) was used to determine whether the older sibling differed from the younger sibling with respect to age or year of onset.

The two clinic populations were analysed separately and together (*see* Results). All analyses were considered significant at the 5% level.

Description of subgroups used in the data analyses. The nontwin sibling population was arbitrarily divided into the following 3 subgroups: (1) siblings who are 1–3 yrs apart in age (subgroup I); (2) siblings who are 4–8 yrs apart in age (subgroup II); (3) siblings who are more than 8 yrs apart in age (subgroup III). Concordant monozygotic twins were analysed separately.

Data analysis using linear regression (*see* fig. 2). Differences in age of onset and year of onset, for each of the subgroups I to III as well as for the total nontwin sibling pair group, were analysed by linear regression. Fig. 2 summarizes the expected results according to 3 hypotheses previously outlined.

Intraclass correlation (*see* fig. 2). An intraclass correlation (Zar, 1974) to compare ages of onset between and within the families was done for the nontwin sibling pairs (subgroups I–III and for the entire group) as well as for the monozygotic twins. If ages of onset show significantly more variation between families than within families, a correlation of onset ages between siblings within a pair is implied. A genetic hypothesis would predict an intraclass correlation coefficient (r) significantly greater than zero. The degree of correlation could be close to 1 in a single gene model and be less correlated if polygenes influence age of onset independent of susceptibility. Conversely, the random exposure hypothesis would predict that onset ages would vary as much within a family as between families, resulting in no correlation (*see* fig. 2).

Clinic comparisons. We compared the data using the same analyses from the two separate clinics (London and Vancouver) as independent data sets to see if there were any differences between the populations.

Anticipation. Onset in the second individual to be affected within a family can lead to earlier recognition of symptoms and diagnosis. To evaluate the possibility of this phenomenon we determined the time from first symptom to diagnosis and Kurtzke disability scores in the first 37 sibling pairs encountered in the London clinic.

RESULTS

Study population

Ninety-nine sibling pairs were identified from the MS Clinics in London, Ontario (n = 51) and Vancouver, British Columbia (n = 48). Details on each individual pair are presented in the Appendix. Subgroups I, II and III by clinic are given in Table 1. The 198 individuals from the 99 sibling pairs consisted of 119 females and 79 males for a female to male ratio of 1.5:1. This ratio is slightly lower than the observed rates

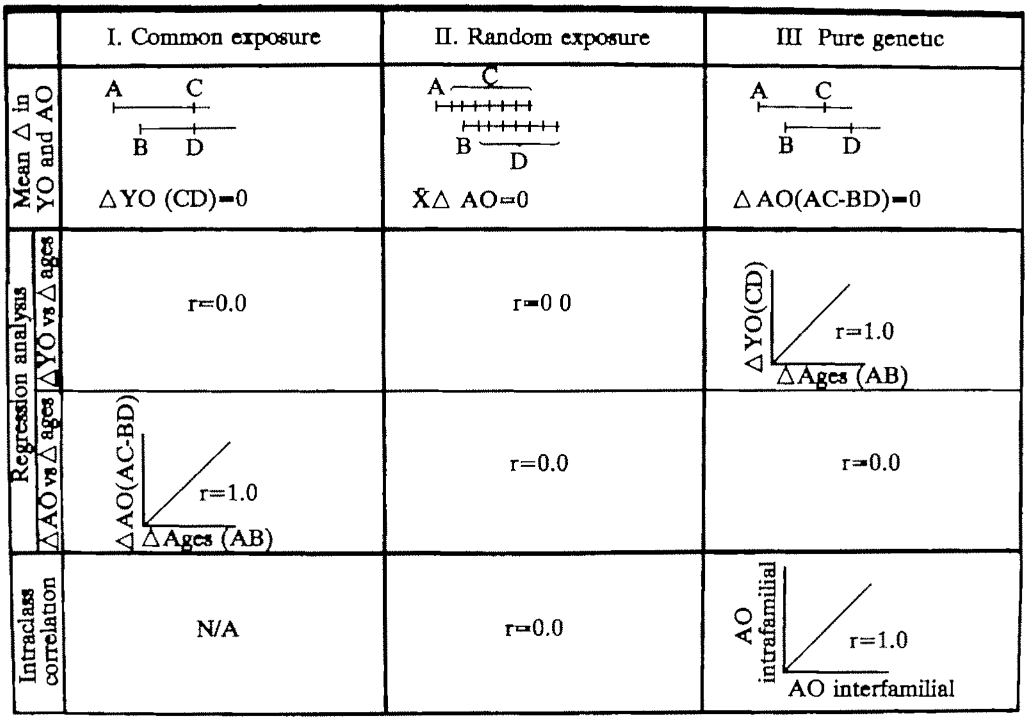

FIG. 2. The hypothetical results predicted by: I, common exposure, II, random exposure, and III, pure genetics hypotheses. Hypotheses I and III predict that the difference in year or age at onset, respectively, will equal zero. Hence, when the mean age or year at onset is calculated for the entire group of siblings, the value will also equal zero. Hypothesis II predicts that the difference in age at onset will vary randomly between pairs of concordant siblings Accordingly, examination of the entire group of siblings should result in a mean difference in age at onset of zero. YO = year of onset; AO = age at onset. For further explanation, *see* fig 1

TABLE 1 NUMBER OF SIBLING PAIRS IN EACH SUBGROUP BY CLINIC

*Subgroup**	*MS Clinic London*	*MS Clinic Vancouver*	*Both clinics*
I	19	17	36
II	20	23	43
III	12	8	20
Total	51	48	99

* Subgroups I, II, III consist of sibling pairs who differ in age by 1–3, 4–8, and >8 years, respectively.

of 1.8:1 and 2:1 for the overall MS clinic populations in London and Vancouver, respectively (unpublished data). Sibling pairs from each clinic were found to be very similar with respect to mean differences in ages, age of onset, and year of onset (Table 2) and were therefore combined for subsequent analysis.

TABLE 2. MEAN DIFFERENCE (SD) IN AGES, YEAR OF ONSET AND AGE OF ONSET BETWEEN SIBLINGS WITHIN A PAIR IN YEARS

MS clinic	*n*	*Ages*	*YO*	*AO*
London	51	5.61 (3.44)	2 29 (13 41)	3.33 (13.18)
Vancouver	48	5 35 (3.32)	3 94 (11.82)	1 42 (11.86)

YO = year of onset. AO = age of onset.

Analysis of mean differences in age and year of onset

The mean differences in ages, year of onset and age of onset were determined for sibling pairs in subgroups I, II and III as well as for the entire sibling pair population. Results are shown in Table 3. The mean differences in age and year of onset in subgroups I and II did not differ significantly from zero. For subgroup III the older siblings tended to have the onset of MS before the younger sibling, with the mean difference in year of onset being significantly greater than zero (t = 4.660, $P < 0.001$) and identical to the mean difference in ages. Furthermore, Table 3 shows that only for subgroup II did the mean difference in age of onset differ significantly from zero (t = 3.196, $P < 0.005$).

TABLE 3. MEAN DIFFERENCE (SD) IN AGES, YEAR OF ONSET AND AGE OF ONSET IN YEARS IN THREE GROUPS OF CONCORDANT SIBLING PAIRS

		Mean difference in		
*Subgroup**	*n*	*Ages*	*YO*	*AO*
I	36	2.22 (0.68)**	2.75 (13.01)	−0.56 (13.17)
II	43	5.81 (1.50)**	−0.14 (12.07)	6.00 (12.31)**
III	20	10 65 (2.01)**	10 65 (10.22)**	0.00 (12 20)
Total	99	5 48 (3.36)**	3.09 (12.63)**	2.40 (12.53)

* Subgroups I, II, III consists of siblings who differ in age by 1−3, 4−8, and >8 years, respectively ** Not equal to 0 00; $P < 0.05$ (actual value in text). YO = year of onset, AO = age of onset.

In the total study group of 99 siblings, there was a mean difference in year of onset of 3.09 yrs, which was significantly greater than zero (t = 2.4343, $P < 0.02$). The older sibling therefore developed MS on average 3 yrs before the younger sibling. The mean difference in age of onset was 2.40 yrs, not significantly different from zero (t = 1.9058, $P > 0.05$).

Onset analysis by linear regression

For subgroup I, the mean difference in year or age of onset did not differ significantly from zero. The resulting correlation coefficients of the differences in age and year of onset for each sibling pair were r = 0.158 ($p > 0.20$) and r = −0.106 ($P > 0.50$), respectively.

For subgroup II, the mean difference in year of onset was not significantly different from zero. Regression analysis as for subgroup I showed a correlation coefficient (r) of 0.244, not significantly greater than zero ($P > 0.10$). The difference in year of onset vs the difference in ages failed to show a significant correlation; ($r = -0.129$, $P = 0.20$). Similarly, the mean difference in age of onset for subgroup III was not significantly different from zero. The correlation coefficient for the difference in year of onset vs the difference in ages for subgroup III also did not differ significantly from zero ($r = 0.109$, $P > 0.50$). The difference in age of onset vs the difference in ages was $r = 0.087$, $P = 0.50$.

Overall, the entire group of 99 sibling pairs had a mean difference in age of onset which was not significantly different from zero. The resulting correlation coefficient for the difference in year of onset vs the difference in ages was $r = 0.165$, $P > 0.10$. The difference in age of onset vs the difference in ages was $r = 0.106$, $P = 0.20$.

Intraclass correlation

Intraclass correlations for age of onset (*see* Table 4) for subgroups I, II, and III as well as for the entire population showed the following: subgroup I gave a significant correlation ($r_1 = 0.299$, $F = 1.854$, $P < 0.025$), whereas subgroups II and III did not ($r_1 = 0.152$, $F = 1.360$, $P > 0.10$; and $r_1 = 0.267$, $F = 1.729$, $P > 0.10$, respectively). Age of onset within sibships showed a modest but significant correlation for the entire group of 99 sibling pairs ($r_1 = 0.234$, $F = 1.610$, $P < 0.01$). Intraclass correlation of the 7 concordant monozygotic twin pairs yielded a highly significant correlation ($r_1 = 0.847$, $F = 12.095$, $P < 0.005$).

TABLE 4. INTRACLASS CORRELATION OF AGE AT ONSET IN FOUR GROUPS OF SIBLING PAIRS CONCORDANT FOR MS

Sibling pairs	*n*	*Correlation coefficient*	*Probability*
Monozygotic twins	7	0.847	$P < 0.005$
Subgroup I (1–3 yrs apart in age)	36	0.299	$P < 0.05$
Subgroup II (4–8 yrs apart in age)	43	0.152	$P > 0.10$
Subgroup III (>8 yrs apart in age)	20	0.267	$P > 0.10$
Total of nontwin sibs	99	0.234	$P < 0.01$

Comparison of clinics

In Table 2 the sibling pair data for each clinic is given separately. For the London clinic, the mean difference in age of onset is 3.33 (1.42 for the B.C. clinic), while the B.C. clinic showed a mean difference in year of onset of 3.94 (2.29 for London).

Anticipation

For the first of a sibling pair to be diagnosed, the mean time from onset to diagnosis was 3.8 yrs (SD = 5.5) compared with 3.8 yrs (SD = 5.7) for the second to be diagnosed. The mean Kurtzke Disability Score at diagnosis was 2.9 (SD = 1.5) and 2.5 (SD = 1.2) in the first and second sibling to be diagnosed, respectively.

DISCUSSION

There are several constraints on the use of age of onset data to make inferences about the relative importance of genetic and environmental influences on the development of MS. First, biological onset probably precedes clinical onset in many patients by some years, although at present there is no way to identify biological onset. Secondly, the precise definition of clinical onset depends on patients' recall. This may become less accurate with time, tending to make the reported onset either later or earlier than the actual onset. For this reason, we routinely used medical records to corroborate reported clinical onset. Thirdly, ascertainment of sibling pairs concordant for MS may be biased towards an earlier age of onset since sibling pairs presently discordant for MS might be expected to include a higher proportion of those whose MS would begin at a later age. Fourthly, the age of onset in a second sibling may be influenced by the phenomenon of 'anticipation' whereby a second affected individual within a sibship comes to attention earlier because of familiarity with the symptoms of the disease. We have evaluated the possibility of anticipation by comparing time to diagnosis from first symptom for the first sibling to be affected with that for the second affected sibling. The results show no difference. This lack of difference could result from there being no anticipation or a mixture of denial (volitional delay in reporting symptoms) and true anticipation. Our experience with a large number of familial cases suggests that the latter, namely a mixture, may be true. Finally, some homogeneity between and within affected pairs is assumed although this is by no means proven. The lower male to female sex ratio for the sibling pairs has been found in a large study of familial vs nonfamilial cases consistent with predictions for polygenic inheritance of susceptibility (Weinshenker *et al.*, 1990).

The ideal way to study sibling pairs would be to identify a cohort of population-based MS patients and their siblings, follow them to an age at which the risk of developing MS would be negligible, e.g., age 60 yrs (Ebers, 1986) and then identify all concordant pairs. However, it is not feasible to do such a study at this time. Our data could be confounded by a relative deficiency of sibling pairs in which the second sibling to be affected has a late age of onset. The mean difference in age of onset might be reduced then to the extent that the first born sibling is the first to develop symptoms more often than the second (a modest tendency seen in both Schapira's data (1963) and our own). This could increase the difference in year of onset to approach more closely the difference in ages. This potential bias could alter the data only slightly. Furthermore, birth order in multiply affected sibships has been examined previously (Cripps *et al.*, 1982) and was found to be random for affected pairs within a sibship. The possibility that time to clinical onset (incubation period) could be influenced by gender and/or age at exposure to a hypothetical environmental trigger has only been considered as it might affect intraclass correlation. As noted, this seems unlikely to have much effect (group I and group III being similar).

Finally, the mean age of onset is slightly younger for females compared with males (by 1.4 yrs on average for 1200 patients in the London, Ontario data; personal unpublished results). This could affect the expected difference in age of onset in unlike-sex sibling pairs. We have not corrected for this influence because for the first 40 pairs ascertained, a correction of 1.5 yrs was made and did not alter the results (data not shown) since in approximately one-half of such pairs the female was the first to develop MS symptoms.

Means

Sibling pairs close in age are a priori the least informative for differentiating between common exposure, random exposure, and genetic determination, as onset would be expected to occur both at the same age and during the same year, according to hypotheses 1 and 3. Similarly, siblings far apart in age could be the most informative about common exposure, as they would be expected to share the least number of common environmental experiences.

Schapira *et al.* (1963) proposed that if MS were genetically determined, affected sibling pairs should have their onset at the same age. Conversely, if MS onset reflected common exposure to an environmental agent followed by a constant incubation period (which would have to have been non-age dependent), there would be a tendency for MS onset to occur in siblings during the same calendar year. The data of Schapira *et al.* on mean differences showed a trend for the disease to onset in the same year in affected pairs but this was not statistically significant. Using similar methodology, we find the results of mean differences in age of onset (2.4 yrs) and year of onset (3.09 yrs) for the entire group of 99 sibling pairs to be inconclusive. It is to be pointed out that differences in age and year of onset are not independent and one can be derived from the other by knowing the differences in age within any sibling pair. Nevertheless, the expected result with the pure genetic hypothesis might be that the mean difference in age of onset of affected pairs would be zero (observed = 2.40 yrs, not significantly different from that predicted). On the other hand, environmental determination by common exposure would be expected to show a mean difference in year of onset of zero (observed = 3.09 yrs). The latter is significant ($P < 0.05$) and therefore supports the 'pure genetic' hypothesis for the total group of sibling pairs.

When the data for sibling pairs were analysed according to age differences (subgroups I–III) the results were as follows. For subgroups I and II, the mean difference in year of onset did not differ significantly from zero suggesting that these siblings tend to have the onset of MS in the same calendar year. However, the age difference between sibling pairs in these groups is small and thus the least informative. Conversely, for siblings greater than 8 yrs apart in age (subgroup III), the mean difference in age of onset was zero while the mean difference in year of onset approximated to the difference in ages. For the overall group, the mean difference in year of onset (3.09 yrs) was significantly greater than zero with the older sibling developing MS some 3 yrs before the younger sibling. The mean difference in age of onset (2.40 yrs) was not significantly different than zero. It might be argued that the data comparing mean differences are exactly what would result from a 'pure genetic' hypothesis which suggests that affected sibling pairs in group III and in the overall group tend to get the disease at the same age. However, this is misleading. The data from subgroup III could also result from the distribution of ages of onset in the first sibling to be affected being similar to the distribution of the second sibling being affected resulting in a mean difference of zero. This demonstrates the limitation of using differences and means as a measure of genetic vs common exposure influences on the age of onset. If the ages of onset tended to be the same in *individual* pairs, regression analysis should show a correlation between difference in year of onset and difference in ages. Furthermore, intraclass correlation (*see below*) should be the highest in this group. These latter analyses therefore are much more informative.

Linear regression

Linear regression represents another method of evaluating the relationship between age and year of onset in sibling pair subgroups I, II and III. Our initial report on 42 sibling pairs (Cripps and Ebers, 1982) was barely significant in showing a correlation between the difference in age of onset and the difference in ages. By adding additional pairs, the significance was lost and led us to re-evaluate the hypothesis by incorporating the Vancouver clinic data set. No significant result was found using linear regression to analyse difference in age and year of onset vs difference in ages for either the total data set or for any of the subgroups. However, the data do suggest a trend for the onset of MS to occur at about the same age, since the best correlation was between difference in year of onset and difference in ages ($P > 0.1$). The limitations posed by analysis of means are well illustrated by the mean difference in age of onset being zero for subgroup III in the absence of any correlation using linear regression analyses. These findings would be consistent with random independent exposure to a triggering event.

Intraclass correlation

The most direct method to analyse age of onset within specific groups is the intraclass correlation. It would be inappropriate to use an intraclass correlation to examine variation within and between families for year of onset as some siblings did not have the potential to develop MS within the same year. For example, in some cases the age difference between siblings was such that one sibling developed MS symptoms before the birth of the second sibling who eventually was also affected. Accordingly, this analysis was not carried out.

We did use intraclass correlation to compare the variance in age of onset for concordant monozygotic twins, for concordant sibling pairs as a group and for 3 subgroups divided by differences in sibling ages. The intraclass correlation was highest for the concordant monozygotic pairs ($P < 0.005$), less in subgroup I ($P < 0.05$) and decreased as the differences in ages among siblings increased ($P > 0.10$) (subgroups II and III, Table 4). The differences in intraclass correlation among subgroups I, II and III, although modest, raise the possibility of heterogeneity. The nonsignificant intraclass correlations for subgroups II and III imply that age of onset in these groups may largely be determined by random environmental events. Subgroup I (because of the proximity in sibling ages) would presumably contain more 'common exposure' pairs, although the correlation is only modest and not inconsistent with many of these pairs also having had random independent exposure. Nevertheless, the intraclass correlation of age of onset for all sibling pairs is statistically significant.

On average the 3 subgroups of sibling pairs are expected to share the same number of genes. The groups only differ from one another by their difference in ages, yet a difference in age of onset variance is demonstrated. It therefore follows that the increasing correlations could reflect the increased sharing of environment as pairs approach one another in age. It is acknowledged that this effect is modest and not statistically significant.

If we consider that concordant monozygotic twins and concordant sibling pairs have had the same common exposure, it can be argued that the marked difference in correlation between monozygotic twins on the one hand and sibling pairs on the other reflects genetic control of age of onset. Furthermore, the similarity in intraclass correlation between group I ($r = 0.299$) and group III ($r = 0.267$) implies that the difference in sibling ages at the time of exposure has little, if any, effect on the age of onset. In the case of random exposure, no intraclass correlation would be expected except in so far as the sharing of genes influencing age of onset is greater between affected sibling pairs than between affected individuals randomly taken from the pool of affected siblings. These observations are reminiscent of the findings in hereditary disease such as Friedreich's ataxia where intrafamilial correlation of age of onset is greater than interfamilial correlation, presumably on the basis of modifier effects or some genetic heterogeneity (Winter *et al.*, 1981).

Since the monozygotic concordant pairs show a very high age of onset correlation, it could be argued that this reflects a purely genetic influence. However, since intraclass correlation is somewhat greater for siblings closest in age, it is possible that part of the strong monozygotic twin correlation results from environmental sharing. It can be argued that this is a much weaker effect than the genetic influence since the correlation for monozygotic twins is much stronger than for concordant siblings close in age.

We acknowledge that the analyses performed on this sibling pair sample are relatively simplistic and that combinations of genetic and environmental influences (each of which can be heterogeneous) easily confound the data. We concede to some uneasiness about pooling data from 2 populations. However, only modest differences in the mean difference of age and year of onset were found. We cannot exclude the existence of some difference in MS triggers between the 2 populations. However, this difference is only encountered in the comparison of means and we have shown that the use of means is misleading and probably of little value. However, it is clear that the results do not fit well with the notion of a single transmissible agent (Kurtzke and Hyllested, 1979). We feel that the most likely explanation of the results is that MS sibling pairs reflect a mixture of random exposure and common exposure pairs with the former seemingly much more common. It is quite possible that no single MS trigger exists. Rather, several different environmental exposures such as viral infections among others may serve as triggers for the development of autoimmunity as is the case with acute disseminated encephalomyelitis (Alvord, 1985) and postinfectious polyneuritis (Asbury *et al.*, 1969).

In conclusion, the analysis of age and year of onset in MS sibling pairs gives little or no support for the existence of a transmissible agent resulting in a common exposure. The data from monozygotic twins and sibling pairs do support an important genetic influence on age of onset and suggest that the putative environmental trigger is usually a random independent event.

Summary

The findings of this study can be summarized as follows. (1) There are many limitations in using age of onset data for discriminating between genetic and environmental factors. The variance of age of onset in affected nontwin sibling pairs is large. (2) The analysis of mean differences in age or year of onset (Schapira *et al.*, 1963) in sibling pairs concordant for MS can be misleading. Intraclass correlations and linear regressions are more appropriate methods for analysing age of onset data. (3) Linear regression of differences in year of onset or age of onset vs difference in ages showed no significant correlation for the entire population nor for 3 sibling subgroups divided by differences in ages. (4) Intraclass correlations for age of onset showed that concordant monozygotic twins demonstrate a marked tendency to have the same age of onset. If a common trigger is assumed for monozygotic twins and sibling pairs that are concordant, it is implied that age of onset is under partial genetic control. (5) The total population of nontwin sibling pairs tended to have the same age of onset (intraclass correlation, $P < 0.01$).

This is more apparent for sibling pairs close in age (1 – 3 yrs, $P < 0.025$) but did not reach statistical significance for sibling pairs differing by 4 – 8 yrs or more than 8 yrs in age ($P > 0.10$). This supports an important genetic influence on age of onset and is consistent with other data indicating that genes influence susceptibility. (6) Age of onset correlations in the overall group of concordant nontwin sibling pairs are consistent with random independent environmental triggers for most pairs.

ACKNOWLEDGEMENTS

The authors would like to thank Dr T. P. Seland, University of Calgary, Alberta, and Dr R. Nelson, University of Ottawa, Ontario, for providing information on 6 patients. Support for Mr Bulman was provided by The Multiple Sclerosis Society of Canada. Dr Ebers is a Career Scientist with the Ontario Ministry of Health. Dr Sadovnick is supported in part by The Multiple Sclerosis Society of Canada, B.C. Division.

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(*Received July 28, 1989. Revised June 5, 1990. Accepted June 14, 1990*)

APPENDIX

Ninety-nine sibling pairs concordant for MS

	Younger sibling				*Older sibling*				*Differences*		
Family no.	*Sex*	*Yr of birth*	*AO(y)*	*YO(y)*	*Sex*	*Yr of birth*	*AO(o)*	*YO(o)*	*AO(o)-AO(y)*	*YO(y)-YO(o)*	*YB(y)-YB(o)*
197	M	1935	34	1969	M	1934	22	1956	−12	13	1
12	M	1936	36	1972	M	1935	32	1967	−4	5	1
1550	F	1947	20	1967	M	1946	29	1975	9	−8	1
24	F	1948	25	1973	F	1947	21	1968	−4	5	1
6	M	1943	30	1973	F	1942	21	1963	−9	10	1
1199	F	1930	35	1965	F	1928	51	1979	16	−14	2
1036	F	1911	57	1968	M	1909	35	1944	−22	24	2
32	M	1919	57	1976	M	1917	25	1942	−33	34	2
101	F	1944	19	1963	F	1942	22	1964	3	−1	2
257	F	1927	50	1977	F	1925	23	1948	−27	29	2
40	F	1931	40	1971	F	1929	53	1982	13	−11	2
143	F	1941	30	1971	F	1939	17	1956	−13	15	2
51	F	1927	36	1963	M	1925	24	1949	−12	14	2
145	F	1919	35	1954	M	1917	50	1967	15	−13	2
27	M	1930	29	1959	M	1928	45	1973	16	−14	2
1033	F	1948	32	1980	F	1946	31	1977	−1	3	2
1218	M	1934	32	1966	F	1932	35	1967	3	−1	2
1149	F	1960	20	1980	F	1958	24	1982	4	−2	2
2	F	1935	26	1962	M	1933	38	1972	12	−10	2
146	F	1918	25	1943	F	1916	26	1942	1	1	2
22	F	1938	13	1951	F	1936	20	1056	7	−5	2
10	F	1946	24	1970	F	1944	20	1964	−4	6	2
13	M	1937	31	1968	M	1935	28	1963	−3	5	2
18	M	1939	29	1968	M	1936	17	1953	−12	15	3
400	F	1924	40	1964	M	1921	36	1957	−4	7	3
161	M	1932	25	1957	M	1929	30	1959	5	−2	3
11	M	1945	32	1977	M	1942	26	1968	−6	9	3
58	F	1915	48	1963	M	1912	42	1954	−6	9	3
19	F	1920	41	1961	F	1917	52	1969	11	−8	3
39	F	1937	31	1968	M	1934	28	1962	−3	6	3
1051	F	1926	20	1946	F	1923	57	1980	37	−34	3
17	F	1942	21	1963	M	1939	26	1965	5	−2	3

APPENDIX (*continued*)

	Younger sibling				*Older sibling*				*Differences*		
Family no.	*Sex*	*Yr of birth*	*AO(y)*	*YO(y)*	*Sex*	*Yr of birth*	*AO(o)*	*YO(o)*	*AO(o)-AO(y)*	*YO(y)-YO(o)*	*YB(y)-YB(o)*
386	F	1937	29	1966	F	1934	30	1964	1	2	3
31	F	1953	20	1973	F	1950	28	1978	8	−5	3
59	M	1928	37	1965	M	1925	37	1962	0	3	3
29	F	1913	55	1968	M	1910	44	1954	−11	14	3
324	F	1924	40	1964	M	1920	36	1956	−4	8	4
1200	F	1925	53	1978	M	1921	43	1964	−10	14	4
114	F	1926	20	1946	F	1922	27	1949	7	−3	4
28	F	1950	28	1978	F	1946	25	1971	−3	7	4
49	F	1945	23	1968	M	1941	22	1963	−1	5	4
35	F	1914	18	1932	M	1910	61	1971	43	−39	4
291	M	1928	41	1969	F	1924	39	1963	−2	6	4
1037	M	1929	16	1945	F	1925	19	1944	3	1	4
36	M	1936	35	1971	M	1932	33	1965	−2	6	4
14	F	1955	15	1971	F	1951	27	1979	12	−8	4
57	F	1959	21	1980	F	1955	16	1969	−5	11	4
108	F	1952	23	1975	F	1948	24	1972	1	3	4
281	M	1919	29	1948	M	1914	23	1937	−6	11	5
60	M	1946	23	1969	M	1941	40	1981	17	−12	5
384	M	1927	33	1960	M	1922	18	1940	−15	20	5
228	M	1929	46	1975	M	1924	48	1972	2	3	5
7	M	1929	29	1958	F	1924	23	1947	−6	11	5
26	F	1940	23	1963	F	1935	30	1965	7	−2	5
46	F	1946	33	1979	M	1941	26	1967	−7	12	5
191	F	1948	19	1967	F	1943	30	1973	11	−6	5
216	F	1906	32	1938	F	1900	42	1942	10	−4	6
1040	M	1949	31	1980	M	1943	34	1977	3	3	6
1145	F	1948	20	1968	F	1942	32	1974	12	−6	6
1035	F	1947	30	1977	F	1941	26	1967	−4	10	6
64	F	1935	20	1955	M	1929	46	1975	26	−20	6
9	M	1922	19	1941	F	1916	55	1971	36	−30	6
33	M	1942	25	1967	M	1936	31	1967	6	0	6
4	M	1930	45	1975	M	1923	41	1964	−4	11	7
144	F	1934	29	1963	M	1927	32	1959	3	4	7
1047	F	1952	28	1980	F	1945	32	1977	4	3	7
1039	M	1955	25	1980	F	1948	29	1977	4	3	7
1543	F	1934	30	1964	F	1927	20	1947	−10	17	7
1799	F	1944	37	1981	F	1937	48	1985	11	−4	7
3	F	1923	39	1962	F	1916	43	1959	4	3	7
42	F	1936	28	1964	F	1929	48	1977	20	−13	7
1547	F	1951	22	1973	F	1943	36	1979	14	−6	8
1	F	1949	19	1968	M	1941	31	1972	12	−4	8
1038	M	1951	28	1979	M	1943	24	1967	−4	12	8
56	M	1928	37	1965	M	1920	60	1980	23	−15	8
1351	F	1947	15	1962	M	1939	45	1984	30	−22	8
1542	F	1946	34	1980	F	1938	40	1978	6	2	8
25	M	1931	36	1967	M	1923	42	1965	6	2	8
30	F	1926	23	1949	F	1918	31	1949	8	0	8
5	F	1939	24	1963	F	1930	21	1951	−3	12	9
15	F	1932	26	1958	F	1923	45	1968	19	−10	9
41	F	1936	43	1979	F	1927	25	1952	−18	27	9
1042	F	1954	20	1974	M	1945	26	1971	6	3	9
23	M	1950	22	1972	F	1941	29	1970	7	2	9
54	F	1941	30	1971	F	1931	34	1965	4	6	10
1157	F	1948	29	1977	F	1938	19	1957	−10	20	10

APPENDIX (*continued*)

Family no	*Younger sibling*				*Older sibling*				*Differences*		
	Sex	*Yr of birth*	*AO(y)*	*YO(y)*	*Sex*	*Yr of birth*	*AO(o)*	*YO(o)*	*AO(o)-AO(y)*	*YO(y)-YO(o)*	*YB(y)-YB(o)*
1052	M	1941	22	1963	F	1931	35	1966	13	−3	10
8	F	1921	49	1970	F	1911	42	1953	−7	17	10
200	F	1917	28	1945	M	1907	17	1924	−11	21	10
37	F	1940	27	1967	M	1930	29	1959	2	9	10
1147	F	1942	35	1977	M	1932	25	1957	−10	20	10
34	M	1935	31	1967	M	1925	45	1971	14	−4	10
1757	F	1949	27	1976	F	1939	20	1959	−7	17	10
16	F	1948	31	1979	M	1937	27	1964	−4	15	11
38	F	1944	30	1974	M	1933	34	1967	4	7	11
20	F	1958	21	1980	F	1946	25	1972	4	8	12
50	M	1946	25	1971	M	1933	18	1951	−7	20	13
21	M	1945	34	1979	F	1931	24	1955	−10	24	14
82	F	1955	12	1967	F	1938	26	1964	14	3	17

AO(o) = age of onset of MS, older sibling, AO(y) = age of onset of MS, younger sibling; YO(o) = year of onset of MS, older sibling; YO(y) = year of onset of MS, younger sibling; YB(o) = year of birth, older sibling, YB(y) = year of birth, younger sibling.

Brain (1991), **114**, 951–978

A NEUROPHYSIOLOGICAL COMPARISON OF THREE DISTINCT REGIONS OF THE PRIMATE FRONTAL LOBE

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SUMMARY

We examined whether reported physiological distinctions between prefrontal (PF) and premotor (PM) areas reflect true dissimilarity or instead result from technical considerations such as differences among species, individuals, or behavioural, physiological and anatomical techniques. A Rhesus monkey performed a task involving 2 stimulus events on each trial: the first stimulus appeared briefly, then after a delay period, 2 peripheral stimuli appeared simultaneously. The monkey had to delay responding for an additional, self-timed *instructed delay period*, then contact a touch pad beneath the peripheral stimulus of the colour matching the first stimulus.

PM and PF cortex differ in several respects. More PF than PM neurons discharge during the presentation of the first stimulus, and only PF neurons show phasic bursts of activity shortly after that stimulus disappears. During the instructed delay period, cells in PF begin activity earlier than PM neurons, whereas PM neurons continue their activity longer. Similarly, PF cells show greater discharge modulation early in the instructed delay period compared with PM cells, which discharge more intensely later. Cells with apparent selectivity for stimulus characteristics appear only in PF. Thus our results support the view that PM and PF have distinctive physiological properties that cannot be accounted for by technical considerations alone.

In addition, we describe the activity cells in a cortical region, here termed the dorsomedial frontal cortex, which has a distinctive dysgranular cytoarchitecture and has physiological properties that ally it with PF.

INTRODUCTION

The motor and prefrontal areas of the primate frontal lobe have long been considered functionally distinct, a view that originated with architectonic studies in the first part of this century (Brodmann, 1905, 1909; Campbell, 1905; Vogt and Vogt, 1919; von Economo, 1929; Bucy, 1935) and has been supported by behavioural and clinical studies (Milner, 1964, 1982; Luria, 1980; Freund and Hummelsheim, 1985; Passingham, 1985, 1988; Stuss and Benson, 1986; Goldman-Rakic, 1987; Fuster, 1989; *see also* Fulton, 1934). Recent neuroanatomical evidence (Barbas and Pandya, 1987, 1989; Arikuni *et al.*, 1988; Cavada and Goldman-Rakic, 1989*a*, *b*; Preuss and Goldman-Rakic, 1989; Ungerleider *et al.*, 1989) generally supports the older studies in promoting this dichotomous conception of frontal lobe organization.

Published physiological data from nonhuman primates also suggest a functional distinction between prefrontal areas, on the one hand, and the frontal motor areas, on the other. In one scheme, PF is specialized for attending to, analysing and temporarily

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storing sensory, spatial and temporal information, whereas PM and other motor areas are specialized for planning responses (Fuster, 1985*a*, 1989). We will term this idea the canonical view. As expressed recently by Fuster (1989, p. 124):

> 'the processing of motor action seems to involve, in succession, progressively more posterior frontal regions, from prefrontal, through premotor, to motor cortex. Accordingly, the nerve cells in that anteroposterior sequence of cortical regions show progressively less coupling to sensory stimuli and more coupling to movement. . . . Presumably, the prefrontal cortex, the highest stage of that frontal processing hierarchy, represents the action in broad outline and prepares the motor apparatus for it; premotor and motor areas implement the more concrete aspects of the action.'

As attractive as such a synthesis may be, the potential remains for substantial difficulty. Different experimental designs, different species of nonhuman primates, different laboratories, and nonidentical cortical regions have been involved in past work. Indeed, there are only a few, limited studies that explicitly compare the activity in a motor area and a prefrontal area (Kubota and Funahashi, 1982; Vaadia *et al.*, 1986; Watanabe, 1986*a, b*, 1990; *see also* Sakai, 1978; Pragay *et al.*, 1987). The canonical view is based upon a quantitative assessment of the relative proportions, in different frontal regions, of neurons with multitudinous properties. For the most part, three classes of neurons are most prominent in prefrontal areas: (1) cells that respond to behaviourally important visual or auditory stimuli, (2) cells active immediately before a movement, and (3) cells active tonically during various behavioural delay periods (Fuster and Alexander, 1971; Kubota and Niki, 1971; Fuster, 1973, 1985*b*; Niki, 1974*a, b, c*; Sakai, 1974; Niki and Watanabe, 1976, 1979; Kojima, 1980; Kubota *et al.*, 1980; Watanabe, 1981, 1986*a, b*, 1990; Ito, 1982; Kojima and Goldman-Rakic, 1982, 1984; Kubota and Funahashi, 1982; Thorpe *et al.*, 1983; Vaadia *et al.*, 1986; Joseph and Barone, 1987; Sawaguchi, 1987; Quintana *et al.*, 1988; Barone and Joseph, 1989; Funahashi *et al.*, 1989*a*). Similar activity patterns, at least by qualitative analysis, can be observed in premotor cortex (Kubota and Hamada, 1978; Godschalk *et al.*, 1981; Weinrich and Wise, 1982; Watanabe, 1986*a, b*; Kurata and Wise, 1988; Crammond and Kalaska, 1989; Riehle and Requin, 1989) and in other motor areas (Tanji and Evarts, 1976; Kwan *et al.*, 1981, 1985; Wannier *et al.*, 1989). The canonical view holds that the aforementioned areas differ in the relative proportions of neurons in classes 1 and 3, above, in the proportion of cells that combine class 1 and 2 activity, and in the timing of their onsets and offsets in relation to sensory cues (class 1) and motor acts (class 2). Such quantitative comparisons are tenuous when they involve data from a variety of individuals and species studied with an assortment of behavioural and anatomical methods.

Alternatively, some authors have emphasized qualitative differences between prefrontal and frontal motor areas. Parts of PF are said to reflect in their activity motivation, reinforcement or related matters (Rosenkilde *et al.*, 1981; Thorpe *et al.*, 1983; Ono *et al.*, 1984; Inoue *et al.*, 1985; Yajeya *et al.*, 1988; Watanabe, 1989). But investigators concentrating on frontal motor areas have typically failed to search for such properties. In other cases, characteristics such as apparent sensory responsiveness have suggested clear, qualitative distinctions between prefrontal and frontal motor areas (Bignall and Imbert, 1969; Sakai, 1978; Pigarev *et al.*, 1979; Komatsu, 1982; Mikami *et al.*, 1982; Suzuki and Azuma, 1983; Azuma and Suzuki, 1984), but other studies show that such properties are not uncommon in motor areas (Kwan *et al.*, 1981, 1985; Rizzolatti *et al.*, 1981; Wannier *et al.*, 1989).

In view of these difficulties, an alternative view of the neurophysiological and neuroanatomical data remains plausible. On that view, reported physiological differences between prefrontal and frontal motor areas may be only apparent and could be due to the many diverse sources of data available. Accordingly, it is important to compare a premotor and a prefrontal area directly, in the same animal, to confirm the distinctions previously suggested. The present project was designed to put the canonical and alternative views to the test.

METHODS

Behavioural methods

A male Rhesus monkey (9 kg) was operantly conditioned to perform a visuomotor task. After the animal adapted to the primate chair, it was seated near a panel containing 3 square touch pads (38.4 cm^2) and 3 bicolour light-emitting diodes (LEDs). The 18×48 cm panel was placed at arm's length in front of the monkey at shoulder level. The touch pads indicated when the animal contacted each pad with the glabrous surface of the left forepaw. The 3 pads and the 3 LEDs were arranged in a horizontal array at arm's length in front of the monkey, as schematically illustrated in fig. 1. The LEDs could emit either a red or a green light, but the 2 signals also differed in luminance, apparent size of the source, and other visual parameters. Other than the LEDs, the room illumination was kept relatively low, but was neither measured nor controlled. After dark adaptation, the monkey could have seen his left forelimb and parts of the apparatus immediately before him. During conditioning, the monkey's right arm was loosely restrained and his head movement was limited by a padded brace that extended around the back of the head toward the snout on both sides.

The monkey initiated each trial by depressing the central of the 3 touch pads (fig. 1A). After a *reference period* of 2.0 s, the central LED was illuminated (the *central stimulus*) for 1.0 s. The central stimulus was randomly selected and was one of two types: (1) a red LED or (2) a green LED coupled with an auditory stimulus (Sonalert). After the central stimulus was turned off, there was a *first delay period* of 1.25 s, followed by the illumination of both the left and right LEDs (the *peripheral stimuli*, also referred to as *cues*). One of the peripheral cues, selected randomly, was red, the other was green. After receipt of the peripheral stimuli, the monkey was required to delay movement for a self-timed period of at least 1.25 s but less than 3.5 s, then move his forelimb to the touch pad immediately beneath the LED of the colour matching the central stimulus on that trial. We did not present a trigger, or 'go' signal. The peripheral cue remained illuminated until the monkey contacted either the left or the right touch pad. If the monkey contacted the correct pad, it received a 0.1 ml juice reward. Otherwise, the monkey received no reward and, in either event, had to return his forelimb to the central pad to initiate another trial. If the monkey broke contact with the central touch pad at any time before the end of the self-timed delay period, all lights turned off and the trial had to be reinitiated. There were no constraints on the monkey's behaviour between trials but, in general, the monkey returned to the central touch pad soon after receiving a reward. The task is a variety of the delayed matching-to-sample task used previously (Rosenkilde *et al.*, 1981; Watanabe, 1981; Fuster *et al.*, 1982; Quintana *et al.*, 1988), in which a small set of stimuli is repeatedly presented. This behavioural method contrasts with most neurophysiological applications of the matching-to-sample task, in which trial-unique stimuli are typically employed.

Recording methods

When the monkey learned the task to a performance level of 90% correct, a stainless steel recording chamber (27 mm×36 mm) was implanted over the right frontal lobe. During the aseptic surgical procedure the monkey was anaesthetized with sodium pentobarbital (30 mg/kg, i.v.) and monitored to ensure adequate anaesthesia. In the same procedure, stainless steel screws and head bolts, which were used to stabilize the head during recording, were implanted in the cranium behind the recording chamber. After a five-day recovery period, during which the monkey received an analgesic (Banamine, Schering Corp., 1.4 mg/kg, i.m.), the daily recording sessions commenced. Platinum-iridium, glass-insulated microelectrodes (1–3 MΩ

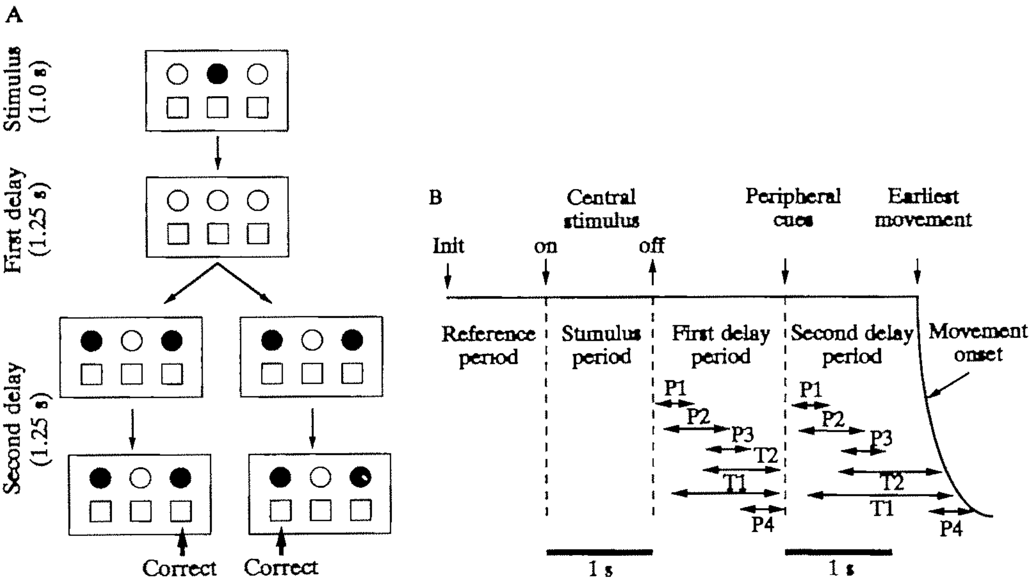


FIG. 1. A, Schematic illustration of the panel. Squares represent the 3 touch pads and circles the LEDs. *From top to bottom*: the central stimulus presentation is indicated by the filled circle (a red light), during the first delay none of the LEDs were illuminated, then 1 of 2 patterns of peripheral cues were presented (green lights are represented by the hatched circles, red by filled circles). The correct response is indicated by the upward pointing arrows for the 2 trial types illustrated here. The other 2 trial types began with a green central stimulus and the correct responses would be the opposite to those indicated in the figure B, temporal sequence of task events and the designation of task periods. The horizontal line indicates the period during which the monkey was required to maintain contact with the central touch pad The sloping vertical line represents the approximate range of movement onset times, which may have begun at any time after the end of the 1.25 s self-timed second delay interval (earliest movement). The horizontal arrows show the classification scheme used for phasic (P) and tonic (T) activity patterns

at 1 kHz), inserted through the intact dura mater into the frontal cortex, were used to monitor neuronal activity. The unit potentials of individual neurons were isolated with a time-amplitude window discriminator (Bak Electronics, DIS-1), which signalled the computer (Digital Equipment Corp., LSI 11/23) each time the neuron discharged an action potential. The time of each discharge was stored in a computer file along with the times of other behavioural events, such as the illumination of LEDs or the monkey's contact with a touch pad. Toward the end of the recording sessions, eye position was monitored with an oculometer (Bouis Electronics), and the positional signal was sampled at 100 Hz, converted to a digital value, and stored along with the other behavioural data. The locus of exploration switched often from one cortical region to another.

Single cell analysis

The activity of each unit was evaluated by visual examination of perievent time histograms aligned on the sensory events and movement onset. Only correct, rewarded trials were analysed. The task epochs were designated as the reference, stimulus, first delay, second delay, and movement periods (fig. 1B). A neuron was classed as task related if it had a significant increase or decrease in discharge rate during a task period compared with the reference period (Mann Whitney U test, two-tailed, $P < 0.05$). Neuronal modulation was classified according to its temporal characteristics. The first and second delay periods were divided arbitrarily into two parts: their first 350 ms and the remainder of the delay period. Phasic neuronal discharge that began and ended in the first 350 ms was classed as P1, cells that began discharging in the first 350 ms of the period but continued into the second were labelled P2, and cells that began and ended in the later subdivision were designated P3. These discharge patterns have been termed signal-related in the past (Weinrich and Wise, 1982; Weinrich *et al.*, 1984). Cells tonically activated during the delay periods were classed as T1 or T2, with the former beginning their discharge in the first 350 ms and the latter beginning afterwards. These tonic discharges have been termed set-related activity (Weinrich and Wise, 1982; Weinrich *et al.*, 1984). Activity at the end of the delay period was labelled P4, and corresponded to anticipatory, or precue, activity (Mauritz and Wise, 1986; Vaadia *et al.*, 1988) in the first delay period, and to movement-related activity in the second.

The timing of neuronal activity changes in relation to most events was determined by comparing task-related neuronal activity in 30 ms bins to activity in the reference period (from 1.5 s to 0.5 s before the onset of the central stimulus). If 2 consecutive bins differed from the reference level by more than 2 SDs of the mean, the onset latency was defined as the beginning of those bins. The offset of neuronal activity was similarly defined as 2 consecutive bins with activity within 2 SDs of the reference period following a larger neuronal modulation. For the relation to the movement onset, comparison was made with the period from 0.8 s to 0.5 s before movement onset, rather than with the reference period. The modulation rate was defined as the absolute value of the difference between the mean discharge rate in the appropriate task period and that in the reference period.

Histological methods

Near the end of physiological data collection, electrolytic lesions (10 μA for 10 s, cathodal current) were made at 7 sites. One week later, the monkey was deeply anaesthetized with sodium pentobarbital (60 mg/kg, i.v.) and, when all cutaneous and corneal reflexes ceased, the animal was perfused through the heart with isotonic saline, followed by 3.0% formaldehyde (w/v) in phosphate (pH 7.4) buffer. During the perfusion, steel pins were inserted at known coordinates. The brain was removed, photographed, sectioned on a freezing microtome at 40 μm thickness, mounted on glass slides, and stained for Nissl substance with 0.25% thionin. Surface projections of the recording sites and the estimated track of each penetration was plotted by reference to the 5 recovered electrolytic lesions and to the pin holes. No attempt was made to identify each recording tract in the histological material or to determine the depth of recording for any cell.

RESULTS

Behavioural data

Six samples of the monkey's behaviour were taken during the 5 months of neurophysiological data collection: 2 early in the series of recording sessions, 2 late, and 2 in the middle. The monkey performed the task with an overall success rate of 96%. There was little difference among the 4 different trial types in percentage correct, reaction time, or movement time. The monkey chose correctly on 92% of trials in which the central stimulus was red and the peripheral cues instructed a rightward limb movement; 97% when a red/left combination occurred; 99% for green/right; and 96% for green/left trials. The monkey's response latencies and movement times were also similar for the various trial types, with leftward movements beginning approximately 20 ms earlier and terminating about 35 ms earlier than rightward movements, regardless of central stimulus colour. There were no behavioural differences that depended on the colour of the central stimulus.

The monkey's oculomotor behaviour was not controlled in the present study, but was sampled and found to be consistent. During the early part of each trial the monkey either continued gazing at the central stimulus location or made a saccadic eye movement to the central stimulus shortly after it appeared. Fixation on the central stimulus was maintained for the 1 s it was illuminated. During the first delay period, the monkey virtually always made a saccadic eye movement to the right, at a latency of 225–275 ms and maintained gaze in approximately the direction of the right cue. During the second delay the monkey made several saccadic eye movements, which consistently reflected the direction of movement instructed rather than the pattern of peripheral cues. For almost every trial, the monkey made a saccade to the left peripheral cue at a latency of 200–275 ms after the onset of the peripheral cues. Then, if those cues indicated a leftward movement on that trial, the monkey saccaded back to the right peripheral cue at an additional latency of approximately 250 ms, and subsequently returned gaze to the left peripheral cue or target until the movement. However, if the cues instructed a rightward movement, the monkey maintained gaze to the left peripheral cue for longer, approximately 500 ms, then fixated the right peripheral cue or target location until the movement.

Neuronal data

The activity of 228 task-related neurons was recorded in 103 penetrations. Samples were taken from 3 cortical regions: premotor cortex (PM), prefrontal cortex (PF), and a cortical region rostral to PM, as usually defined, which we term the dorsomedial frontal cortex (DMF). These regions were distinguished on the basis of surface landmarks and cytoarchitectonics (*see below*). Table 1 shows the number of task-related neurons in each region.

Stimulus period. The number of cells with significant modulation during the stimulus period (fig. 2) and their percentage (of the sample within each cortical region) is given in Table 1. The differences among the three cortical regions are significant ($\chi^2 = 8.05$, df = 2, $P < 0.05$), with the largest number and percentage observed in PF. During the stimulus period, only PF contained cells (n = 7, 7%) that were apparently selective for nonspatial stimulus properties (fig. 3); no such selectivity was observed in either PM or DMF. The 3 frontal regions explored differed little in amplitude, latency or duration of stimulus-period discharge (Tables 2, 3).

First delay period. The 3 cortical regions sampled did not differ in the percentage of task-related units active during the first delay period, when all such activity was considered together. However, differences were observed if finer classifications are drawn: phasic offset responses (including the P1, P2, and P3 classes) were totally absent from PM, though well represented in the other 2 populations, where they make up about half the cells with task-related modulation during the first delay period (Table 1). The differences among the areas were significant at the $P < 0.005$ level ($\chi^2 = 16.8$, df = 4, P1+P2+P3 vs T1+T2 vs P4). Fig. 4 shows an example of a neuron's activity during the first delay period. As with activity during the stimulus period, there were only a few neurons with apparent specificity for stimulus quality, but all 4 were located in PF. There were few, if any, differences among the sampled areas in the timing or amplitude of task-related modulation, regardless of the detailed discharge pattern (Tables 2, 3).

Second delay period. The second delay period commenced with a complex visuospatial stimulus that, together with the stored information from the central stimulus, instructed the monkey about the movement to be made on that trial. Most cells showed significant activity modulation during this instructed delay period, and much of it was directionally selective (figs 5, 6), i.e., activity significantly differed when the stimuli instructed leftward vs rightward movements. The activity of these cells did not reflect the pattern of peripheral

TABLE 1 DISTRIBUTION OF CELL ACTIVITY PATTERNS

Task period	*Activity pattern*	*No. of cells*			*Percentage*		
		PM	*PF*	*DMF*	*PM*	*PF*	*DMF*
Total		55	97	76	100	100	100
Stimulus		6	29	12	11	30	16
First delay	P1, 2, 3	0	13	12	0	13	16
	T1	3	1	1	6	1	1
	T2	8	10	12	14	10	16
	P4	2	6	5	4	6	6
Second delay	P1	3	30	27	6	31	35
	P2	11	40	25	20	41	33
	P3	0	12	8	0	12	10
	T2	15	6	5	27	6	6
	T1	18	3	4	33	3	5
Movement	P4	28	34	19	50	35	25

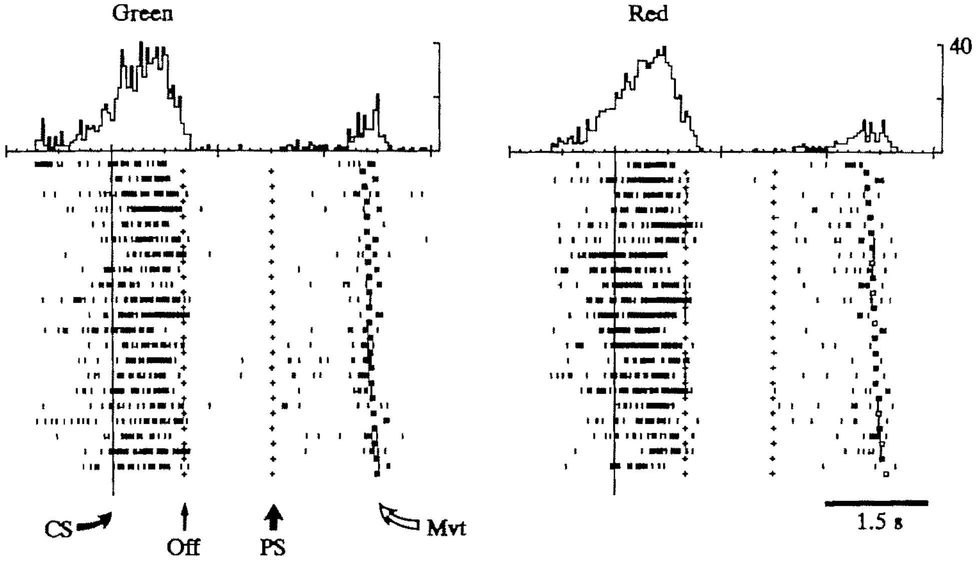


FIG. 2. PM neuron showing tonic discharge during the stimulus period. The trials occurred in a random order, but here they are segregated according to the colour of the central stimulus. Each line of the raster display, beneath the histogram, shows the activity of this neuron during a single trial. Only correct and rewarded trials are shown. Each short vertical mark shows the time that an action potential occurred. Reading each display *left to right*: the long vertical line, upon which the display is aligned, indicates the time that the central stimulus (CS) was illuminated during the trial. It turned off at the time of the first plus sign (beneath each raster line). The next plus sign beneath each line indicates when the peripheral stimuli (PS) were illuminated. The open squares indicate the approximate onset of movement on a given trial The trials are ordered according to the interval between the central stimulus presentation and the onset of movement (Mvt), with shortest interval at the top of each raster display. The activity scale: impulse/s, identical in both displays.

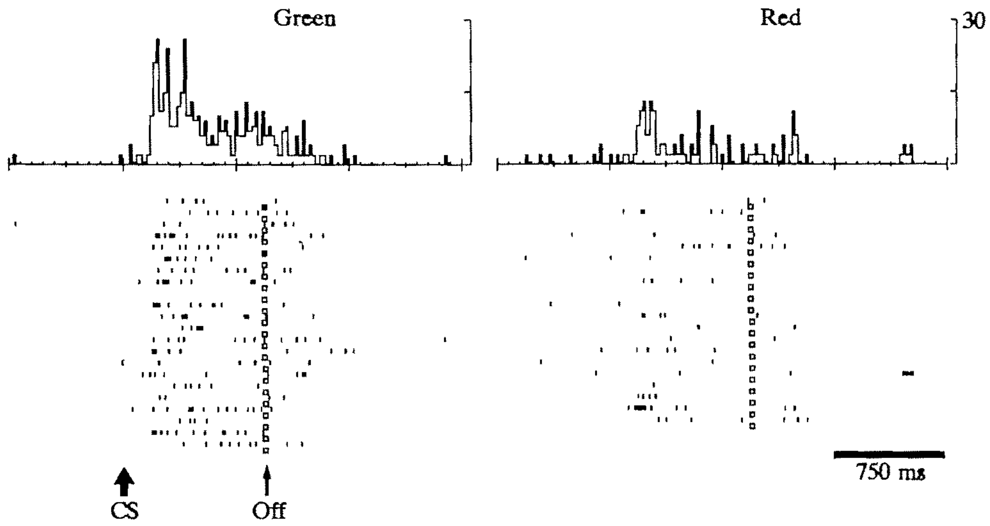


FIG. 3. PF neuron showing apparent selectivity for stimulus characteristics. Note the greater discharge modulation after green central stimuli vs red. Trials are segregated according to the central stimulus colour; abbreviations as in fig. 2.

TABLE 2. LATENCY AND DURATION OF ACTIVITY

	Latency			*Duration*		
Task period	*PM*	*PF*	*DMF*	*PM*	*PF*	*DMF*
Stimulus	127 ± 101	117 ± 49	121 ± 79	443 ± 349*	320 ± 225	326 ± 176
1st delay						
Phasic	—	223 ± 155	311 ± 233	—	235 ± 60	297 ± 102
Tonic	652 ± 262	693 ± 238	677 ± 222	599 ± 263	550 ± 222	573 ± 223
2nd delay						
P1	167 ± 11*	80 ± 33	96 ± 43	160 ± 20	185 ± 73	182 ± 61
P2	215 ± 71**	153 ± 75	160 ± 83	402 ± 180	324 ± 81	316 ± 101
P3	—	441 ± 70	414 ± 65	—	260 ± 258	304 ± 96
T1	170 ± 108	173 ± 117	235 ± 41	1083 ± 154	947 ± 285	960 ± 48
T2	614 ± 119***	543 ± 88	470 ± 37	673 ± 125	697 ± 120	621 ± 137
Phasic and tonic	301 ± 233*	190 ± 158	192 ± 140	762 ± 354*	311 ± 193	326 ± 217
Premovement	−123 ± 91*	−274 ± 122	−209 ± 160	253 ± 105	329 ± 122	274 ± 125

* Differs significantly from both PF and DMF at $P < 0\ 05$; one-factor ANOVA, with multiple post hoc comparisons used to compare each region with each of the other two regions (Scheffé F test). ** Differs significantly from PF but not from DMF. *** Differs significantly from DMF, but not from PF

cues (green to the left/red to the right, or vice versa). Such directional selectivity occurred in similar proportions in all 3 frontal regions: 72%, 62%, and 69% in PM, PF, and DMF, respectively. In the second delay period, when PM and DMF cells showed selectivity for the different trial types, it was always selective for the direction of response, and not for stimulus properties (*see*, e.g., fig. 2). By contrast, 10 of 97 PF cells (10%) had significantly greater activity for movement toward the red (6 neurons) or green (4 neurons) peripheral stimuli, regardless of the direction of movement.

TABLE 3 AMPLITUDES OF ACTIVITY (MODULATION) DURING THE DIFFERENT TASK PERIODS (|MEAN PEAK−REF|)

		Modulation of cells in each cortical area		
Task period		*PM*	*PF*	*DMF*
Reference level		9±7	5±5	6±4
Stimulus		53±18	33±24	38±18
First delay	P1−3	—	20±10	23±7
	T1−T2	24±18	23±12	44±27
Second delay	P1	19±5**	45±41	42±23
	P2	34±19	35±26	26±17
	P3	—	24±12	25±12
	T2	30±22	21±11	16±11
	T1	49±40***	32±14	26±13
Premovement	P4	61±55*	32±18	21±20

* Differs significantly from both PF and DMF. ** Differs significantly from PF at $P < 0.05$, but does not differ from DMF; one-factor ANOVA with multiple post hoc comparisons (Scheffé F test) *** Differs significantly from DMF at $P < 0.05$ but does not differ from PF.

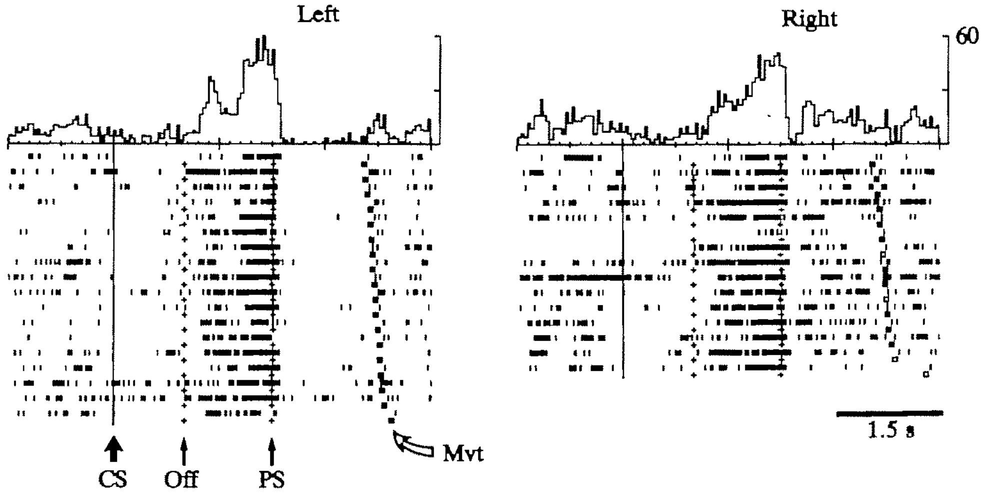


FIG. 4. DMF neuron active during the first delay period, but also showing modulation during the second delay period (between PS and Mvt). Note that in this latter period there is inhibition before leftwards trials. Note also that the activity during the first delay period does not differ according to the direction of movement. Format as in fig. 2, except that trials are segregated according to movement direction rather than central stimulus colour.

Eighty-four percent of PF, and 78% of DMF, task-related neurons display phasic discharge patterns during the second (instructed) delay period (Table 1, fig. 7). This contrasted strongly with the characteristics of PM (Table 1), in which relatively few

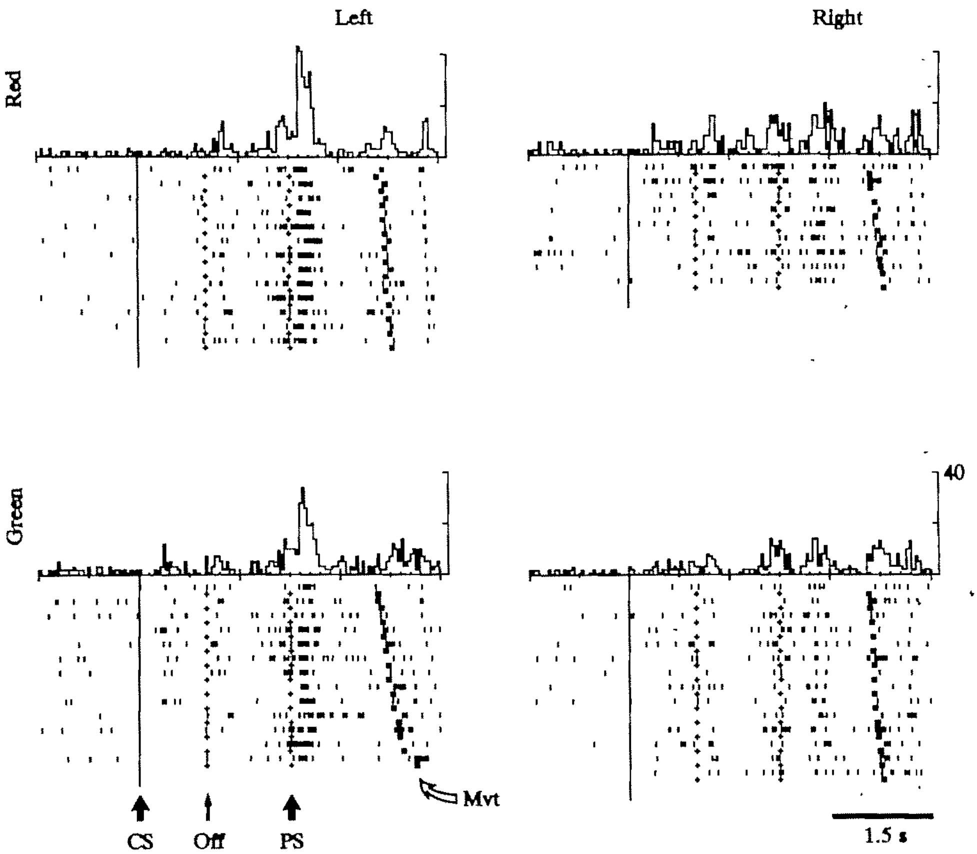


FIG. 5. PF neuron showing a directionally selective response following the peripheral stimuli. The 4 raster and histogram displays show activity for each of the 4 trial types separately, although they occurred in random order. The *top row* shows trials in which the central stimulus was red, the *bottom row*, green. The *left column* shows trials that required a leftward forelimb movement to achieve a reward, the *right column*, rightward movements Note that displays on the diagonal have identical peripheral cues. Format as in fig. 2.

neurons (26%) showed phasic activity, and especially few showed early, brief (P1) or later (P3) phasic bursts. In PM, there were instead many cells with tonic changes in activity during the second delay period (figs 7, 8). These differences were significant ($\chi^2 = 100.0$, df = 6, $P < 0.001$) and depended on the contrast between PM, on the one hand, and PF and DMF on the other: the latter 2 regions did not show significant differences in activity patterns during the second delay ($\chi^2 = 0.71$, df = 3, $P > 0.87$).

Similar distinctions were observed in the amplitude of discharge. Cells classed as having the P1 activity pattern were not only fewer in PM, but had significantly lower modulation levels compared with PF. Cells with significant modulation later in the delay period, namely T2 and P4 cells (*see* fig. 1B) had larger modulation amplitudes in PM, whereas in PF and DMF, the earlier P1 activity had the highest modulation (Table 3). Thus the movement-related (P4) modulation in PM exceeded that in PF and/or DMF

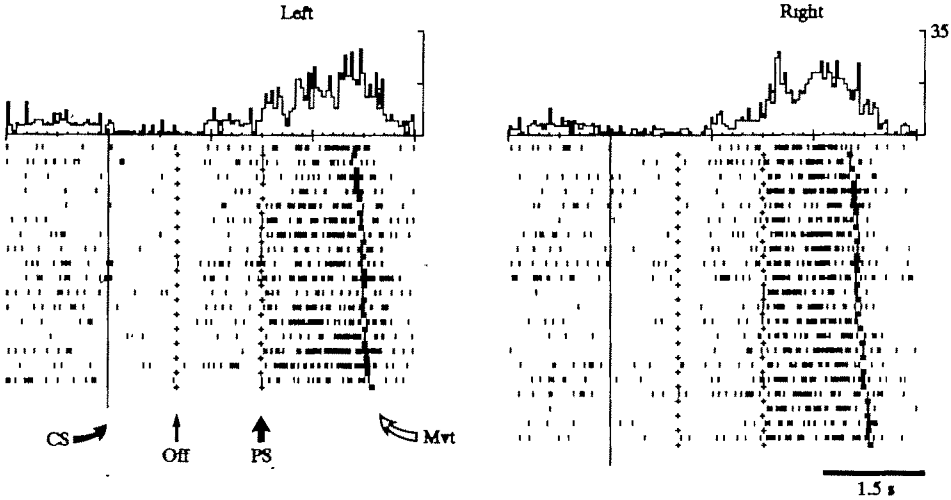


FIG. 6. PF neuron with trials grouped according to the direction of limb movement. This cell shows tonic discharge during the second delay period, in which the monkey has been instructed about the direction of limb movement to be made on that trial, but is withholding movement pending the elapse of a self-timed 1.25 s delay period. This cell also shows a phasic burst of activity following the peripheral stimuli, which is greater when the stimuli indicate that a rightward movement should be executed than when they instruct a leftward movement. Format of each raster and histogram display as in fig. 2.

(and is the greatest for any class of PM neuron), whereas the phasic modulation was greater in PF and DMF than in PM (and was the greatest for any class of PF or DMF neuron).

The latency of phasic discharge in PM exceeded that in PF and/or DMF, for both phasic (P1) and tonic (T2) activity (Table 2, fig. 7). As for the duration of activity, 70% of PM neurons had discharge durations in excess of 500 ms, whereas only 11% of PF cells and 19% of DMF cells had such long activity durations. This difference can also be appreciated from the population activity during the second delay period

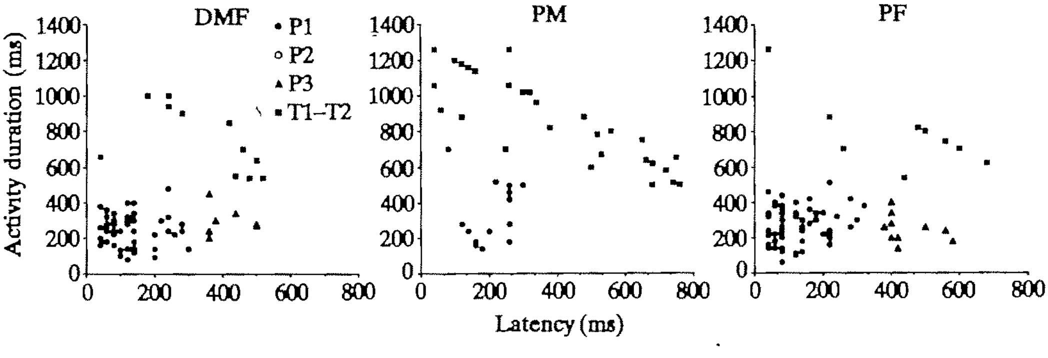


FIG. 7. Scatter plots of activity duration vs latency from the second delay period. The cells were classified according to these 2 parameters into phasic (P1, P2, P3) and tonic (T1, T2) activity. Note the prevalence of phasic cells in DMF and PF, and of tonic cells in PM.

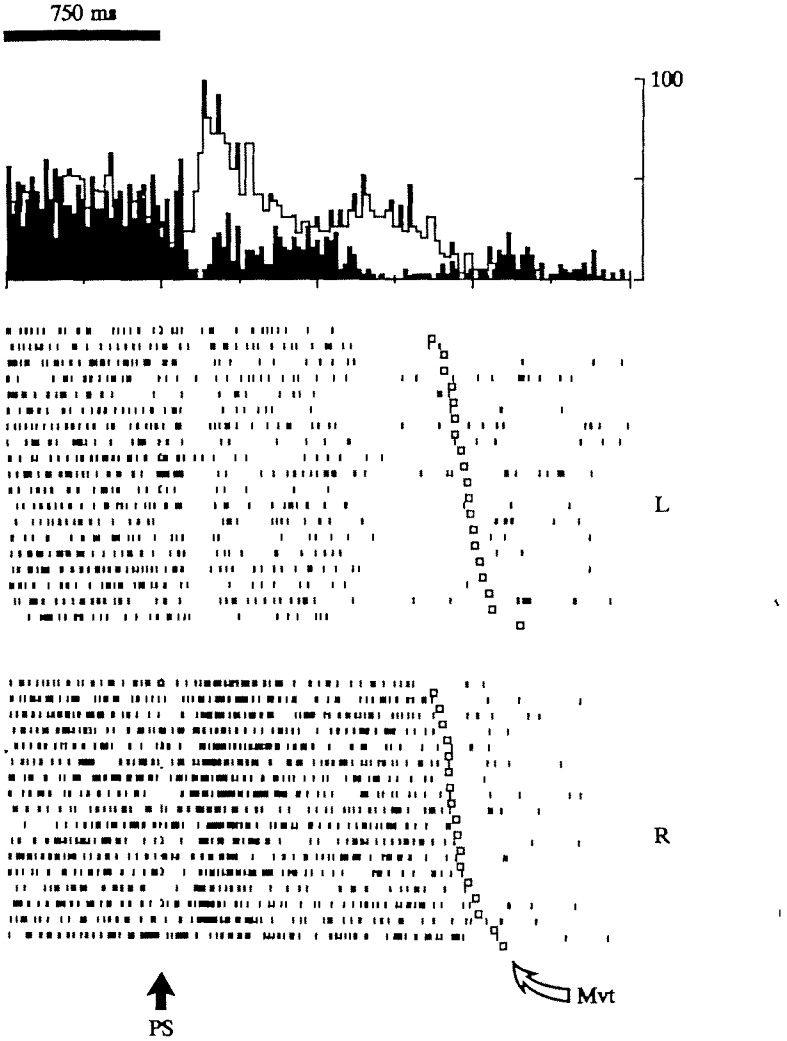


FIG. 8. Tonic PM neuron during the second delay period Activity average from leftward-movement trials is shown with the filled histogram, for rightward movement with the open histogram. Note that there is much greater activity before rightward than before leftward movements.

(figs 9, 10). PM activity began significantly later than that in either PF or DMF in relation both to latency after the peripheral stimulus (figs 9A, 10) and the time before movement onset (fig. 9B) (Kolmogorov-Smirnov test, two-tailed, $P < 0.01$). By contrast, although the PM population activity increased later than that in the other areas, its cells, as a population, continued discharging longer (fig. 10). Similarly, the time to peak discharge was significantly different ($P < 0.05$, Scheffé F test) in PM compared with PF and DMF: in PM the cells' discharge peaked in 661 ± 281 ms, in PF it did so in 273 ± 161 ms, and in DMF, 293 ± 150 ms.

Movement period and combinations. A comparison of the proportion of cells showing premovement modulation (*see*, e.g., fig. 11) did not reveal significant differences among the regions explored ($\chi^2 = 5.20$, df = 2, $P > 0.07$). Directional selectivity was observed in all 3 regions: 14 (50%), 27 (79%), and 9 (59%) neurons in PM, PF, and DMF, respectively. Similarly, the duration of movement-period discharge was comparable in the 3 frontal regions. By contrast, the modulation during the movement period was strikingly greater in PM neurons than in PF and DMF cells (Table 3), and the PM cells became active nearer the time of movement onset (Table 2, fig. 9B).

We examined whether cells that showed premovement activity also modulated their activity phasically or tonically during the second delay period. For PM, only 2 of 28 movement-related cells (7%) also responded phasically following the peripheral stimuli. In contrast, 31 of 34 movement-related (91%) cells in PF did so (fig. 11). Conversely, 18 of 28 (64%) PM neurons, but only 1 of 34 (3%) PF movement-related cells, showed tonic discharge during the second delay period. In DMF, 11 of 19 (58%) movement-related cells showed phasic postcue discharge, and 4 of 19 (21%) discharged tonically. These differences are statistically significant at the $P < 0.001$ level ($\chi^2 = 137$, df = 2).

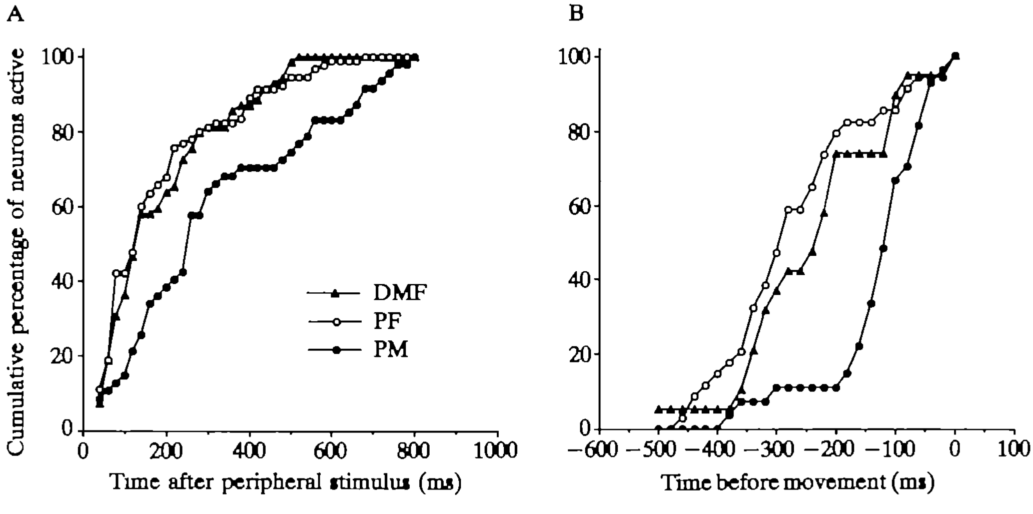


FIG. 9. Cumulative onset times for phasic discharge in the 3 regions A, postcue response latencies Note that the population of PM neurons becomes active later than the DMF and PF populations. B, premovement onset times. Note that the PM population again becomes activated later than the others. DMF (triangles); PF (open circles); PM (closed circles).

Histological data

The cortex explored was divided into 3 regions: the cortex within and immediately ventral to the principal sulcus (PF), the cortex within and medial to the caudal half of the dorsal limb of the arcuate sulcus (PM) and its rostral half (DMF). The cortex in

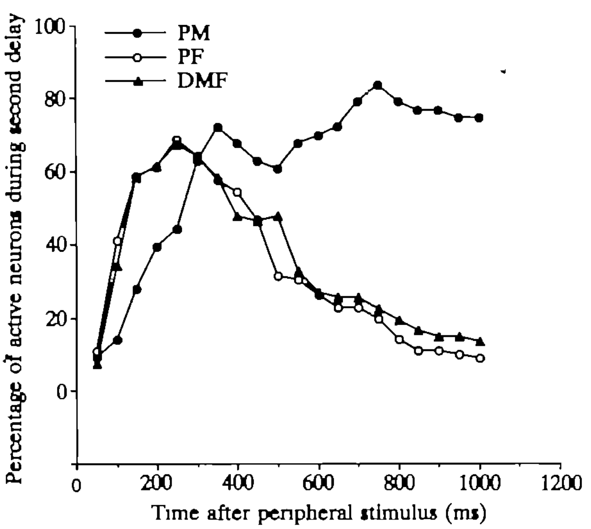


FIG. 10. Profile of the average population activity in each of the 3 regions using a binary addition method. Each neuron is considered 'on' or 'off' according to the criteria detailed in Methods. Activity following the peripheral stimulus. Note that the PM population becomes activated later (*see* fig. 9A), but maintains a high overall activity level longer than either the PF or DMF populations. Symbols as in fig 9.

and near the banks of the principal sulcus has the homotypical cytoarchitecture that has been repeatedly described in the past (Brodmann, 1905; von Bonin and Bailey, 1947; Barbas and Pandya, 1989). The cytoarchitectonic characteristics of the medial frontal gyrus, especially the part nearest the arcuate sulcus, are more varied. Three distinct cortical types could be observed: an agranular zone caudally (fig. 12A), a homotypical zone rostrally (fig. 12C), and a dysgranular zone in between (fig. 12B). We did not record from the homotypical part of the medial frontal gyrus. The dysgranular area corresponds to DMF, the agranular area to PM. The dysgranular region was observed immediately above the superior limb of the arcuate sulcus. It extends 3 mm rostrocaudally, surrounding the tip of the superior limb of the arcuate sulcus, with its mediolateral axis obliquely oriented (fig. 13C). Overall, the cytoarchitectonic characteristics of the dysgranular region are more similar to the caudal agranular cortex, area 6. However, the existence of a tenuous internal granular layer, occasionally broken but nevertheless distinct, delineates the dysgranular cortex from the caudally adjacent agranular region which, in addition, is less clearly laminated. In layer V, the pyramidal cells of the agranular cortex are larger in number and bigger in size than in the dysgranular cortex. There are several differences between this dysgranular region and the rostrally adjacent homotypical cortex (fig. 12B, C): layer IV is denser, thicker and more continuous in the homotypical region, though by no means as well developed as in the periprincipal cortex; in the dysgranular cortex, medium and large pyramidal cells intermingle with granular cells, there are fewer pyramidal cells immediately above layer IV in layer III, and the large pyramidal cells are not as densely packed immediately below layer IV in layer V. Layer VI is thicker in the dysgranular cortex than in the homotypical cortex, either rostral to the dysgranular zone or surrounding the principal sulcus.

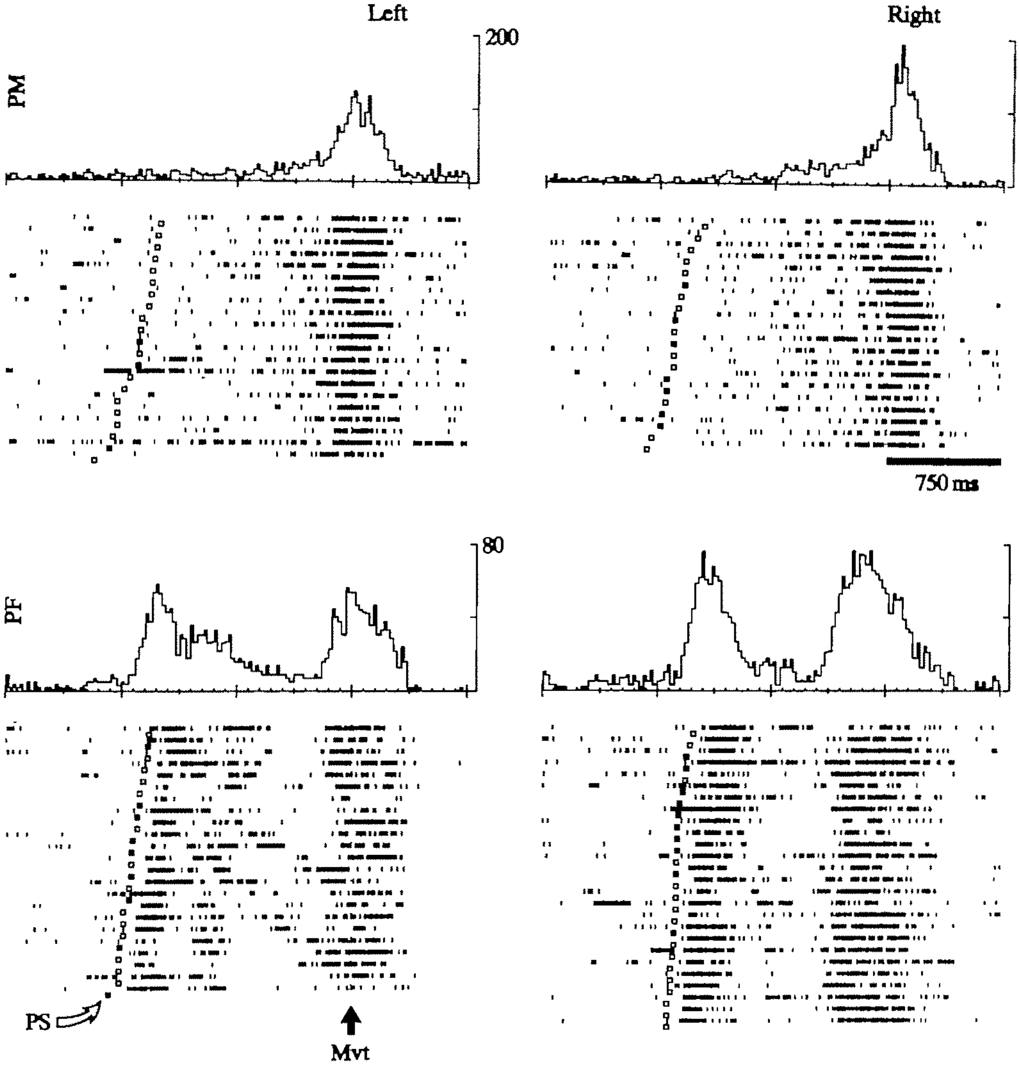


FIG. 11. Movement-related activity in a PM neuron (*top row*) and a PF neuron (*bottom row*). Format as in fig. 2, except that the raster and histogram displays are aligned on the approximate onset of movements. Both neurons show phasic discharge preceding the onset of movement. These cells are representative of the populations in each region in that (A) the PF activity begins earlier and (B) the movement-period modulation is greater in PM. The PF neuron also shows a phasic burst of activity following the peripheral stimuli. Activity scales are identical by row.

The locations of several cell classes are indicated in fig. 13. In viewing these surface plots, the location of the grey matter in the depth of the sulci should be kept in mind. The cortex in the dorsocaudal bank of the arcuate sulcus, where many of the recorded cells were located, is approximately 3 mm thick. Thus cells plotted as within 3 mm of the arcuate sulcus may have been located in its bank (fig. 14). Cells phasically related

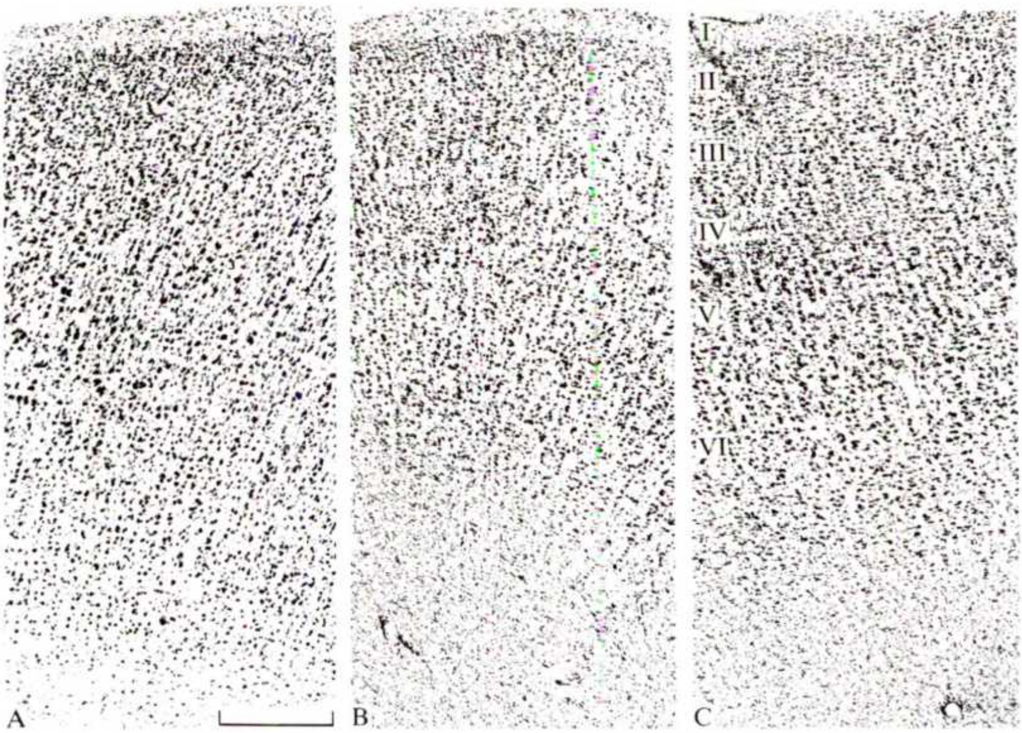


FIG. 12. Photomicrographs of Nissl-stained, coronal sections through the medial frontal gyrus. A, the agranular cortex, area 6, approximately 1 mm caudal to level H in fig. 13C. Magnification bar = 400 μm. B, dysgranular cortex corresponding to DMF, midway between levels D and E in fig. 13C. C, homotypical cortex rostral to the dysgranular zone illustrated in B. Approximately 0.75 mm rostral to level B in fig. 13C, near the mediolateral level of the 'hom' label. Bar = 400 μm.

to the movement (P4, second delay period) can be located in all the regions explored (fig. 13B); there did not appear to be a striking distinction in their distribution compared with the overall locations of task-related cells (fig. 13A). However, clear distinctions were observed in the locations of cells with brief phasic activity bursts (P1) following the peripheral cue (fig. 13C) and neurons showing tonic discharge throughout most of the second delay period (fig. 13D). The former were clearly displaced rostral to the latter, in accord with the population differences described above and in Table 1.

DISCUSSION

The present investigation revealed clear differences between PF and PM that cannot be accounted for by differences in behavioural strategy or by species or individual variation. The neuronal characteristics of a third region, DMF, most resemble those seen in PF. The DMF has a dysgranular cytoarchitecture and, in addition to its (reciprocal) connections with PF (Barbas and Pandya, 1987), DMF also receives a projection from a medial parietal area, PGm, which may provide it with visual inputs (Petrides and

Pandya, 1984; Cavada and Goldman-Rakic, 1989*a, b*). (It should be noted that the current use of the term dorsomedial frontal cortex (DMF) differs from that of Mann *et al.* (1988) who used that term to apply to the supplementary motor cortex and supplementary eye field.) The distinction between PM and DMF does appear, by comparison of surface landmarks, to correspond roughly to the division between area F2 and F7 (part of area 6aβ) by Rizzolatti and his colleagues (Matelli *et al.*, 1986, and personal communication). The dysgranular region corresponds closely with area FC of von Bonin and Bailey (1947). There is a more approximate correspondence of the dysgranular region in the present study with parts of area 6aβ of Vogt and Vogt (1919) and area 6DR as illustrated by Barbas and Pandya (1987).

Comparisons among the regions explored

Anticipatory activity, a tonic or phasic build-up preceding temporally predictable sensory events, was common to all 3 regions. Such activity has been previously described in PM (Mauritz and Wise, 1986; Vaadia *et al.*, 1988), PF (Sakai, 1974; Niki and Watanabe, 1979; Komatsu, 1982; Pragay *et al.*, 1987; Quintana *et al.*, 1988; Boch and Goldberg, 1989) and in other frontal regions, such as the frontal eye field (Bruce and Goldberg, 1985) and the area termed here DMF (Vaadia *et al.*, 1986; Pragay *et al.*, 1987).

Task-related discharge during the second (instructed) delay period was also common to all 3 regions. The earliest reports on neuronal activity in PF concentrated on this delay-period activity (Fuster and Alexander, 1971; Kubota and Niki, 1971; Fuster, 1973; Kubota *et al.*, 1974; Niki, 1974*a, b*). But similar phenomena occur in PM (Kubota and Hamada, 1978; Godschalk *et al.*, 1981; Weinrich and Wise, 1982) and also other motor areas (Tanji and Evarts, 1976; Thach, 1978; Tanji *et al.*, 1980; Georgopoulos *et al.*, 1989). As many as 60% to 70% of PM cells (Wise *et al.*, 1986; Kurata and Wise, 1988; Crammond and Kalaska, 1989) are active during an instructed delay period. In PF, Funahashi *et al.* (1989*b*) found 56% of their cells with instructed delay-period activity (*see also* Fuster, 1973; Fuster *et al.*, 1982; Kojima and Goldman-Rakic, 1982; Batuev *et al.*, 1985;. Watanabe, 1986*a*; Funahashi *et al.*, 1989*a*); Watanabe (1981) found 51%, Fuster (1973) 50%, Niki (1974*c*) 34%, and Kubota and Funahashi (1982) 51%. Of course, these proportions can be affected by many factors, such as the precise behavioural task and the duration of the delay period. Nevertheless, the present results accord with the view that activity during an instructed delay-period is not especially more prominent in any one region than in the others.

In all 3 frontal regions many cells discharge in a directionally selective manner, a finding that conforms with previous reports concerning both PM and PF (Niki, 1974*a, b, c*; Kubota *et al.*, 1980; Watanabe, 1981; Kubota and Funahashi, 1982; Weinrich *et al.*, 1984; Wise and Mauritz, 1985; Kurata and Wise, 1988; Quintana and Fuster, 1989).

Contrasts among the regions explored

Stimulus period. The majority of cells active following the presentation of the central (sample) stimulus were recorded in PF and DMF. Very few neurons in PM exhibited central stimulus-related changes in activity (Table 1). This finding is consistent with selective ablation studies of the inferior convexity of PF (Passingham, 1975; Mishkin

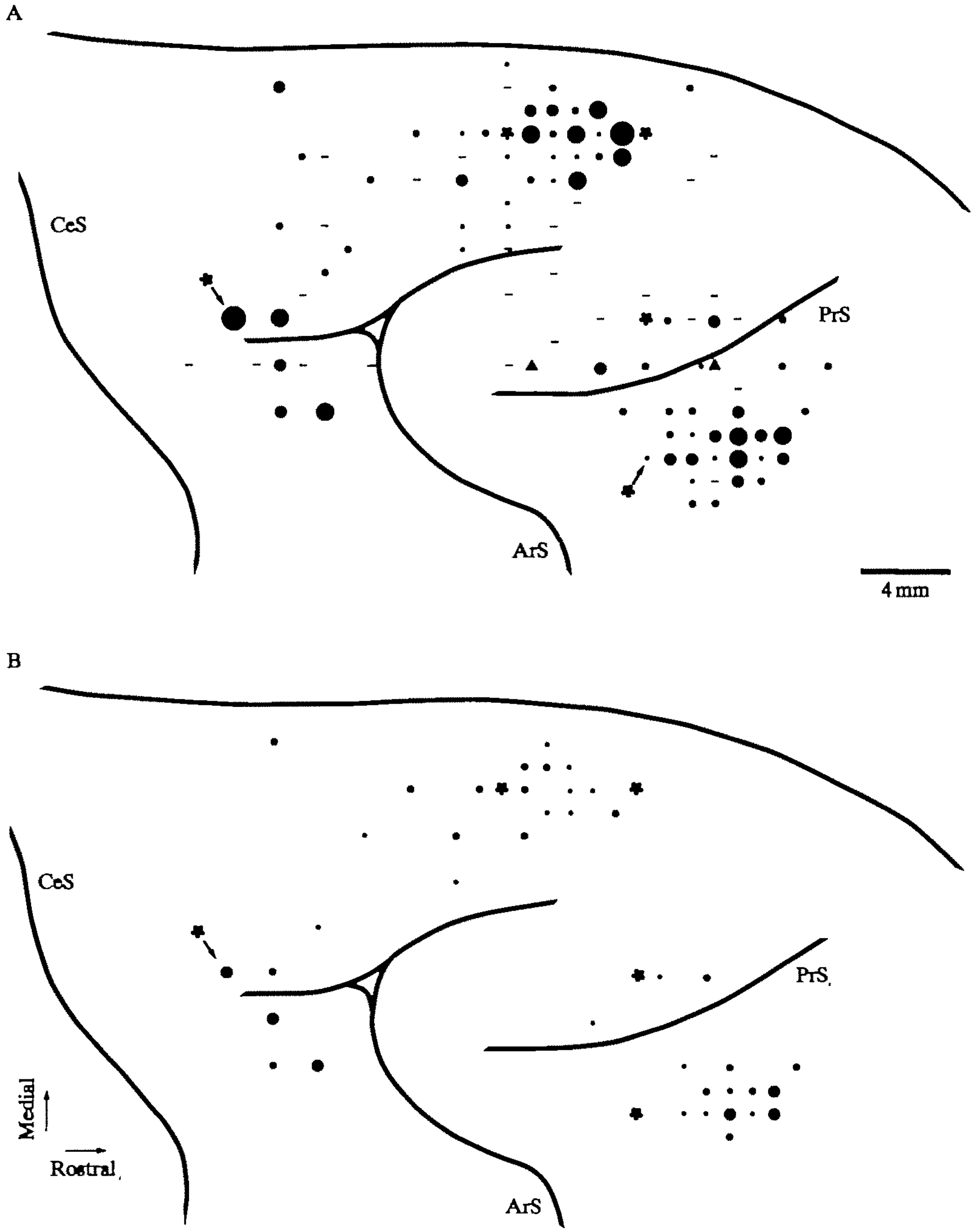
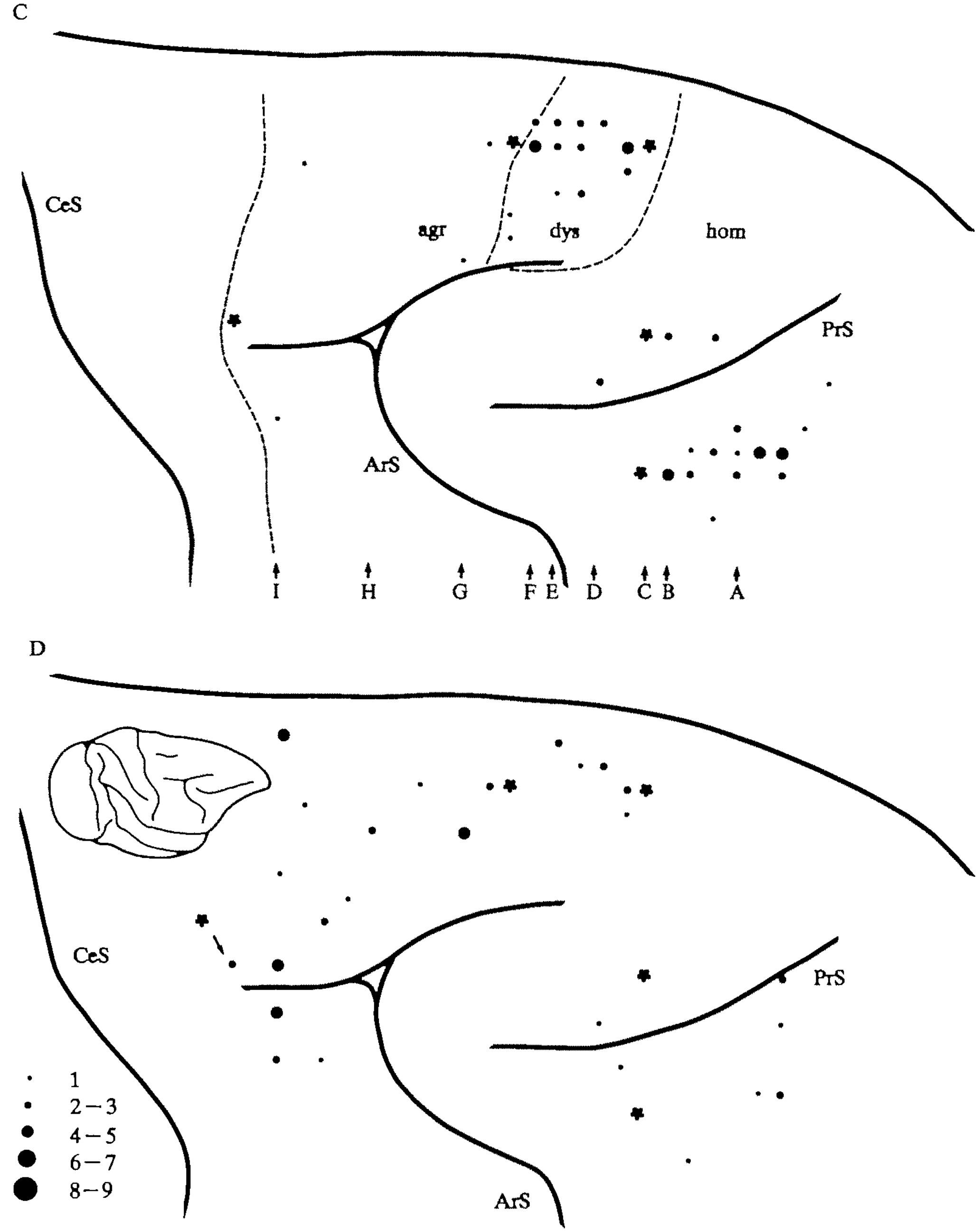


FIG. 13. Surface plots of neuronal locations Each of 4 plots shows the reconstructed location of each penetration based on recovered electrolytic lesions (asterisks) and pins inserted at known electrode coordinates (triangles). The size of each dot indicates the number of neurons in a given penetration with the properties plotted. ArS, arcuate sulcus, PrS, principal sulcus, CeS, central sulcus A, locations of all task-related neurons; dashes show the location of penetrations in which no task-related neurons were observed. B, locations of neurons active before the approximate onset of movement (P4 in fig 1B). Note that these movement-related neurons are distributed in each of the areas sampled.



C, locations of neurons with brief phasic discharges following the peripheral cues (P1 in fig. 1B). Note that these are mostly located dorsal to and within the dorsomedial half of the superior limb of the arcuate sulcus and ventral to the principal sulcus on the convexity of the hemisphere. The 3 dashed lines mark the boundries between areas, with the caudalmost line indicating the boundary between areas 4 and 6. The rostral boundary of area 6 is not shown laterally, but is located near the arcuate sulcus. Upward-pointing arrows refer to the sections in fig. 14. agr = agranular; dys = dysgranular; hom = homotypical D, sites of cells tonically active during the second delay period (T1 and T2 in fig. 1B) Note the more caudal distribution of these cells compared with the phasically activated cells plotted in C.

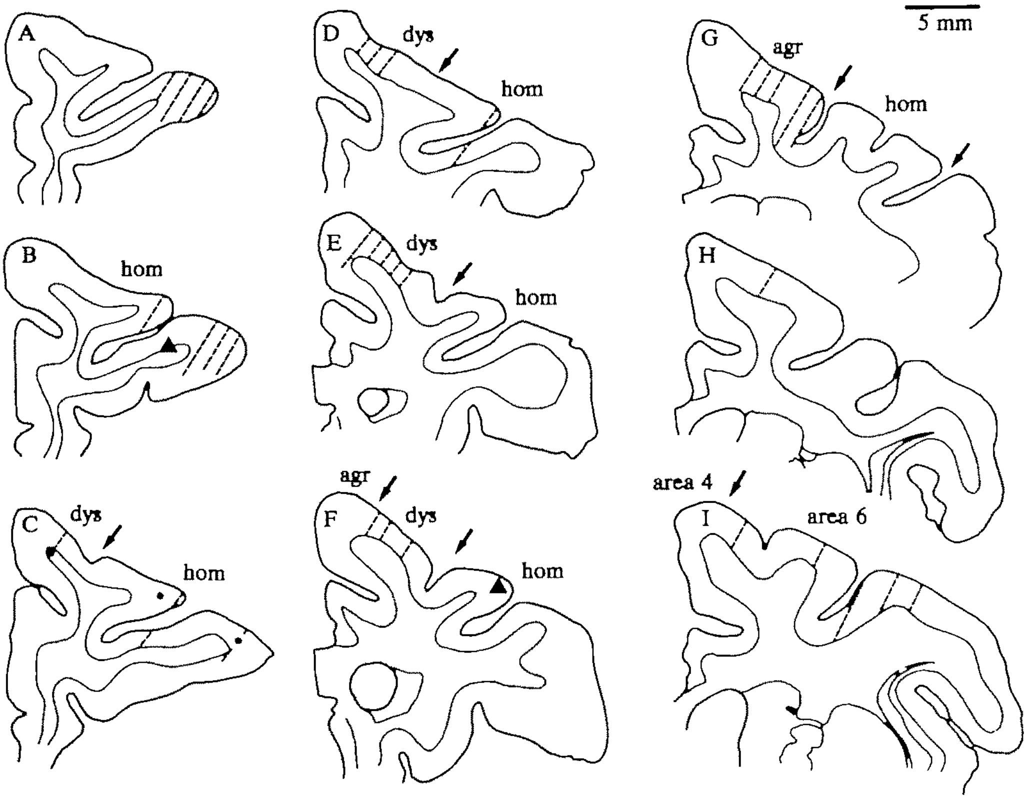


FIG. 14 Frontal sections of the brain showing tracks containing task-related cells, as estimated from the recorded electrode depth. The arrows point to cytoarchitectonic boundaries between the studied regions. The asterisks indicate the locations of 3 of the recovered electrolytic lesions, the filled triangles indicate the sites of the pin holes. Abbreviations as in fig. 13.

and Manning, 1978), where the present PF recordings were concentrated, and neurophysiological data from the same area (Rosenkilde *et al.*, 1981; *see also* Watanabe, 1986*a*), which show that the ventral aspect of the prefrontal hemispheric convexity is necessary for and highly active during delayed matching-to-sample tasks.

Selectivity for stimulus property was not a focus of the present investigation (*cf* Mikami *et al.*, 1982; Suzuki and Azuma, 1983; Azuma and Suzuki, 1984; Bruce, 1988). Nevertheless, we found that only PF had cells with apparent selectivity for the central stimulus. Since the stimuli differed along several visual parameters (colour, luminance, etc.) and one stimulus was bimodal (auditory plus the green central LED), it is impossible to draw any conclusions about what is being 'coded' in the activity of those cells, but findings of this nature have been reported previously for PF (Kubota *et al.*, 1980; Fuster *et al.*, 1982; Watanabe, 1986*a*; Quintana *et al.*, 1988; *see also* Sakai, 1978; Kojima, 1980). For example, in the present task 7% of the PF cells showed 'colour' selectivity during the stimulus period, 4% in the first delay period, and 10% in the second. Likewise, Fuster *et al.* (1982) report 6% of their PF cells that respond to colours per se in the sample period, 5% in the equivalent of our first delay. Kubota *et al.* (1980), in a similar task, reported a value of 9%. The selectivity for stimulus properties in PF, but not DMF or PM, was not unexpected. It has long been known that inferior temporal cortex, which plays a major role in processing visual information concerning colour and form, projects directly to prefrontal areas, particularly to the ventral convexity (Kuypers *et al.*, 1965; Jones and Powell, 1970; Weller and Kaas, 1987; Ungerleider *et al.*, 1989; *see also* Fuster *et al.*, 1985), as does the superior temporal cortex (Petrides and Pandya, 1988).

First delay period. During the first delay period, the monkey had to retain visual information that could not, alone, indicate the response to be made on that trial. The finding that phasic discharge after the offset of the central stimulus (of the P1, P2 and P3 class) was totally lacking in PM, but evident in both other frontal areas examined, is one of the most striking contrasts observed in the present data. All PM modulation during the first delay period is tonic and begins much later than activity in PF and DMF. It is worth noting that the PM discharge following the onset or offset of the central stimulus was much less frequent than the incidence of phasic discharge after the peripheral cues, which served as an instruction for the movement (11% vs 26%). Phasic activity following an instruction stimulus, termed signal-related in the past (Weinrich and Wise, 1982; Weinrich *et al.*, 1984), has been variously suggested to be a visual response or to represent integrative sensorimotor processes. The finding that only a small number of PM cells apparently 'respond' to the central stimulus, which was highly relevant behaviourally, but in itself contained no information about the specific motor act to be made on that trial, supports the latter interpretation of signal-related activity.

Second (instructed) delay period. During the second delay period the differences between PM, on the one hand, and PF and DMF on the other, became most evident. In this study we have fractionated the activity during the second delay period into five classes based on onset latency-duration parameters. We observed that whereas phasic responses predominated in PF and DMF, tonic sustained responses were more prominent in PM. This finding contrasts with that of Funahashi *et al.* (1989*a, b*), who emphasized tonic responses in PF as greater than phasic responses (39–51% vs 17–26%) and Sawaguchi (1987) who found approximately equal proportions (29%) of phasic and tonic discharge. This disparity may stem from differences in classification methods or sampling nonidentical parts of PF: the current PF sample is mainly from the inferior prefrontal convexity, ventral to the principal sulcus and from the ventral bank of the principal sulcus along its caudal half, whereas Funahashi *et al.* and Sawaguchi sampled the cortex around the principal sulcus. However, the present finding agrees with most data from the prefrontal cortex (Fuster, 1973; Kubota *et al.*, 1974; Fuster *et al.*, 1982; Quintana *et al.*, 1988; Quintana and Fuster, 1989). It should be noted that some authors (e.g., Quintana *et al.*, 1988) have used much longer delay periods (18 s) than those used here (1.25 s). Shorter delay periods might decrease the number of PF cells with delay-period activity, and could therefore account for the relatively small number of such cells observed here. The phasic nature of PF activity has been previously mentioned as the most significant difference between PF and primary motor cortex neurons (Kubota and Funahashi, 1982).

One class of phasic cells, P3, was observed in PF and DMF but was not found in

PM. This long latency 'response' has been described in another study of PF, including an area that is probably equivalent to our DMF (Vaadia *et al.*, 1986). The successive activation of P1, P2 and P3 cells during both delays is consistent with a proposal of Batuev *et al.* (1985; *see also* Quintana *et al.*, 1988) of a neural chain as the basis of information storage in the frontal lobe and could reflect a contrast in function of PM vs PF and DMF.

The large proportion of cells with phasic, postcue activity in PF, most of which were directionally selective, is consistent with the inputs this area receives from subdivisions of the posterior parietal cortex such as areas 7a and LIP, which are thought to process visuospatial information (Petrides and Pandya, 1984; Andersen *et al.*, 1985, 1990; Cavada and Goldman-Rakic, 1989*b*). Interestingly, PF also receives direct inputs from extrastriate areas such as the dorsal prelunate (DP) area (Andersen *et al.*, 1990) and the parieto-occipital (PO) area (Colby *et al.*, 1988), as well as from the inferior temporal cortex. On the other hand, dorsal PM, in which we found relatively little phasic, postcue activity, is connected predominantly with area 5 and area 7b, which are largely somatosensory or motor in function.

Movement period. Although less commonly commented upon, studies of PF activity also have reported substantial numbers of neurons with modulation immediately preceding a limb movement (Kubota and Niki, 1971; Kubota *et al.*, 1974; Niki, 1974*a, b, c*; Niki and Watanabe, 1979; Kubota and Funahashi, 1982; Ono *et al.*, 1984; Watanabe, 1986*b*; Quintana *et al.*, 1988). This phenomenon was confirmed in the present study; indeed the differences among the 3 regions in the proportions of such movement-related neurons failed, although barely, to reach statistical significance. A difference of approximately 150 ms was found, however, between the onset times of PM and PF premovement activity (*see also* Kubota and Funahashi, 1982; Watanabe, 1986*b*). Similarly, PM premovement modulation doubled that in PF, and there were differences in the combinations of premovement activity with other activity during the second delay period. Cells that have phasic or tonic discharge both after an instruction stimulus and before a motor response have been repeatedly reported for the frontal areas (Fuster, 1973; Kubota *et al.*, 1974; Niki and Watanabe, 1979; Godschalk *et al.*, 1981; Weinrich and Wise, 1982); however, the areas have not been compared quantitatively. The proportional ratio of PF:PM cells with phasic postcue plus premovement discharge is 13:1. Conversely, for cells with tonic postcue plus premovement activity, the same ratio is 1:21.

The timing differences reported here agree with the view that PM, by virtue of its direct interconnection with the primary motor area, is more closely linked to the motor apparatus than PF and DMF, which project to primary motor cortex indirectly via PM (Matelli *et al.*, 1986; Barbas and Pandya, 1987; Arikuni *et al.*, 1988; Preuss and Goldman-Rakic, 1989).

Interpretational problems

Sampling of neurons is subject to a bias toward larger cells or cells that for whatever reason have large discharge-associated current fields. Thus the finding that a certain percentage of a cell 'class', however defined or identified, differs between 2 cortical regions may reflect differences in the sizes or other electrodynamic properties of those cells. (Of course, inactive cells are rarely monitored and the proportions reported must be understood to apply to actively discharging neurons only.) Despite this limitation, in the absence of any specific reason to presume a systematic cell sampling bias, we assume that our samples offer a reasonable view of the gross, unidentified population activity of the 3 frontal areas. Further work involving the identification of specific classes of cortical projection neurons or other methods will be needed to overcome this limitation (Evarts *et al.*, 1984).

The present results concern 1 monkey only, and it is customary in behavioural neurophysiology to confirm any result in at least a second animal before preparing a report. The present experimental design, however, obviates that requirement by specifically comparing the activity in different areas in 1 individual performing the task in a certain manner and doing so reliably throughout the recording sessions. A regional comparison thus made in 1 monkey is, in our view, preferable to bringing together data from different individuals. Different subjects may perform the same task using unlike strategies, and the vagaries of plotting the locations of recorded single neurons in relation to cytoarchitectonically-defined cortical fields are sufficient to confound comparisons of recording locations from publication to publication.

Finally, because our objective was limited to comparing and contrasting activity of selected frontal cortical regions, and this study did not involve an attempt to interpret the activity in functional terms, we did not concentrate on oculomotor behaviour and recorded no electromyographic activity. Oculomotor behaviour is especially important because some frontal cortex activity is clearly related to the monitoring and/or control of eye movement, and some of our recordings were in the vicinity of the supplementary eye field (Schlag and Schlag-Rey, 1987; Mann *et al.*, 1988; Mitz and Godschalk, 1989; Huerta and Kaas, 1990), or near the principal sulcus, where oculomotor neurons may also be found (Joseph and Barone, 1987; Barone and Joseph, 1989; Funahashi *et al.*, 1989*a*; *see also* Suzuki and Azuma, 1977, but note that the activity reported in that paper could be related to the preparation for lever pressing rather than eye position). As for the supplementary eye field, the areas from which eye movements can be evoked in the medial frontal gyrus appear to be rostrally adjacent to and nonoverlapping with the present recording sites, so it is unlikely that oculomotor interpretations could account for the regional differences observed between DMF and PM. Regarding PF, the preponderance of activity early in the delay periods compared with PM may reflect, in part, a prefrontal role in oculomotor behaviour (Boch and Goldberg, 1989). The monkey made a saccade immediately after the onset of both delay periods. However, the monkey also made one or two saccades during the middle of the second delay period and immediately before movement, but relatively few PF neurons showed activity modulation at those times. Thus it does not appear likely that oculomotor interpretations could completely account for the regional differences observed. It may also be possible that the stereotyped eye movements of the monkey, combined with differential visual receptive field properties among the cortical areas, could cause differences such as we observed. From the limited information so far available concerning PF (Mikami *et al.*, 1982; Suzuki and Azuma, 1983; Boch and Goldberg, 1989) and PM (Rizzolatti *et al.*, 1981) visual receptive fields, there is no reason to assume that their properties could account for the differences reported here.

Conclusions

Despite its long history, the understanding of the functional subdivisions among frontal cortical fields remains unsatisfactory. Problems in cortical field definition within the primate frontal lobe continue to hinder the analysis of both brain-lesion effects and changes in brain activity observed with noninvasive brain-imaging techniques. Future investigation with a variety of neurobiological methods will be required to fully map the primate frontal lobes. Although we have examined only a small part of PF and also only one part of the frontal motor cortex, it appears that the previously reported differences between frontal motor and prefrontal areas are reliable. In general, PF activity begins and peaks earlier in a sensorimotor event; PM activity tends to begin later and becomes greatest later. Thus the present results are consistent with the canonical view outlined in the Introduction and support the hypothesis that, in the selection and control of behaviour, prefrontal areas operate at a hierarchically superior level compared with frontal motor areas.

ACKNOWLEDGEMENTS

The authors thank Drs J M. Fuster, J. Quintana and E. Vaadia for comments on this manuscript.

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(*Received February 20, 1990. Revised June 6, 1990. Accepted June 15, 1990*)

Brain (1991), **114**, 979–999

CNS PATHOLOGY IN THE NEUROLOGICAL MUTANT RATS ZITTER, TREMOR AND ZITTER-TREMOR DOUBLE MUTANT (SPONTANEOUSLY EPILEPTIC RAT, SER)

EXAGGERATION OF CLINICAL AND NEUROPATHOLOGICAL PHENOTYPES IN SER

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SUMMARY

The pathological alterations in the central nervous system (CNS) were examined in three kinds of mutant rat; the zitter (*zi/zi*; Zi), the tremor rat (*tm/tm*; Tm) and the spontaneously epileptic rat (SER) which is a double mutant carrying both zitter and tremor genes. Two major alterations demonstrated in these mutants were hypomyelination and vacuolation or spongy degeneration. Hypomyelination was observed predominantly in SER and to a lesser extent in Zi, and was accompanied by a redundant or aberrant myelin sheath formation in addition to a decreased number of myelinated fibres. This appeared to be related to the occurrence of tremor. There was no abnormality in the structure of the myelin lamellae and oligodendrocytes or any destruction of myelin sheaths by phagocytic cells. The number of radial components in CNS myelin was increased almost equally in Zi, Tm and SER. Vacuolation was prominent in SER and Tm, especially in the brainstem and thalamus. Zi also developed mild vacuolation with advancing age. Vacuolation seemed to be related to the epileptic phenomena in SER and Tm. Vacuoles consisted mainly of swollen astrocytic processes and enlargement of the extracellular space, as well as occasional enlargement of periaxonal spaces. Thus both pathological findings—the hypomyelination derived from the zitter mutation with tremor, and the vacuolation from the tremor mutation with epileptic symptoms—were mutually exaggerated in SER. It is postulated that the two different genetic loci with *zi* and *tm* mutations interact and synergistically reinforce each other both clinically and pathologically in SER.

INTRODUCTION

In 1982, Rehm *et al.* (1982) first reported a mutant rat which manifested generalized spontaneous body tremor and curled hair. It arose in the Sprague-Dawley strain and was designated as zitter (*zi/zi*; Zi). This neurological disorder was inherited in an autosomal recessive manner. Pathological examination of the central nervous system (CNS) was reported as showing spongy degeneration. A few years later, a similar mutant

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rat was found in a colony of Kyo:Wistar strain by Yamada *et al.* (1985). The mutant rat (tremor: *tm/tm*; Tm) also showed curly whiskers and coat hair, and exhibited action tremor. It developed absence-like seizures, and demonstrated spontaneous spike and wave discharges in EEG recordings (Serikawa *et al.*, 1987). The main pathological findings were gonadal atrophy and vacuole formation in the CNS. Subsequently, Serikawa and Yamada (1986) produced a double mutant rat by hybridization between a Zi homozygous female and a Tm heterozygous male. As this double mutant rat exhibited both spontaneous tonic and absence-like seizures, it was named the spontaneously epileptic rat (SER) (Sasa *et al.*, 1988). It has been considered to be a useful model for evaluating the single dose and long-term effects of antiepileptic drugs (Sasa *et al.*, 1988; Serikawa *et al.*, 1990). SER, however, does not survive longer than about 20 wks of age, probably because of status epilepticus and malnutrition. The pathology of the CNS was also characterized by spongy degeneration which was more severe than in Zi or Tm.

There are several reports dealing with the neurophysiological and genetic investigations in these-mutant rats. However, neuropathological reports are rare and, to our knowledge, the pathological findings in the CNS mentioned in these reports have only used light microscopy (Rehm *et al.*, 1982) or have focused on the spongy degeneration in SER (Inui *et al.*, 1990). Furthermore, it remains unclear which pathological alterations are responsible for the tremor and epilepsy in these mutant rats.

Recently, we noticed the presence of significant hypomyelination in these mutants, especially in SER and Zi. In this communication, we have demonstrated hypomyelination in the CNS, including ultrastructural and morphometric analyses of these three kinds of mutants rats, and have elucidated the correlation between the genotypes Zi, Tm and SER, and phenotype manifestations such as clinical symptoms (tremor and epilepsy), neuropathology, and hypomyelination and vacuolation in the CNS.

MATERIAL AND METHODS

A total of 57 rats, 7 SER (*zi/zi, tm/tm*), 12 Zi (*zi/zi*), 9 Tm (*tm/tm*) and 29 control rats which consisted of Sprague-Dawley rats, Wistar rats and some littermates of these mutant rats were examined in this study. These three types of mutant rat and their littermates were maintained and their genetic backgrounds determined as described previously at the Institute of Laboratory Animals, Kyoto University (Yamada *et al.*, 1985; Serikawa and Yamada, 1986).

All animals at various ages (Table 1) were anaesthetized with pentobarbital (50 mg/kg body weight, i.p.), and killed by transcardiac perfusion with 5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4 at room temperature) following a brief rinsing with physiological saline.

TABLE 1. AGE (IN WKS) AND NUMBER OF RATS EXAMINED

Rat	*3*	*12*	*30–34*	*37*	*47*
Control	8	7	5	4	5
Zi	3	3	3	3	
Tm	3	3			3
SER	3	4			

The whole brain, spinal cord, optic nerve and sciatic nerve were removed immediately after perfusion and further fixed by immersion in the same fixative solution for an additional 30–60 min. The upper cervical spinal cord (C1–C2 level), the prechiasmatic optic nerve, and the sciatic nerve (midthigh level) were removed for transverse sections. Other regions such as the cerebral cortex, thalamus, cerebellum, pons, trigeminal nerve, dorsal root ganglia, and the spinal roots were also obtained.

Large blocks were processed by routine paraffin embedding, sectioned at 8 μm and stained with haematoxylin-eosin (HE) or Klüver-Barrera stain. Small pieces of the blocks were postfixed in 1% osmium tetroxide in 0.1 M cacodylate buffer with or without 1.5% potassium ferricyanide for 2 h. The blocks were then dehydrated in a series of graded ethanols, and embedded in epoxy resin via propylene oxide, 1 μm thick sections were stained with toluidine blue for light microscopy. Thin sections from selected areas were stained with uranyl acetate and lead citrate and examined using a Hitachi HU-12AS or H-7000 electron microscope at 75 kV.

For the quantitative analysis of hypomyelination, we selected the optic nerve, the anterior columns of the spinal cord at the C1 – C2 level and the sciatic nerve. Three rats were examined at random from each group at 3, 12, 37 and 47 wks of age. Thick sections were obtained from only those blocks postfixed with 1% osmium tetroxide and 1.5% potassium ferrocyanide to decrease artifactual lamellae splitting. Light microscopic photographs of representative whole transverse sections were taken and the total fascicular area of the optic nerve and the sciatic nerve was measured by a planimeter assisted by a microcomputer. The number or density of myelinated fibres was then calculated from light microscopic photographs of the sciatic nerve and electron microscopic photographs of the anterior columns of the cervical spinal cord and optic nerve, as described in detail previously (Kondo *et al.*, 1986, 1987). The number of glial cells and the aberrant myelin sheaths were also counted per nerve or per 1 mm^2 in these sections.

Electron microscopic photographs at ×13 000 magnification of the sciatic nerve and the anterior columns of the cervical spinal cord, and at ×21 000 magnification of the optic nerve, were used for a correlation analysis between the axon perimeter and the number of myelin lamellae. The axon perimeters were measured by a planimeter and the number of myelin lamellae were counted under a magnifying glass by a method modified from that used previously (Kondo *et al.*, 1988).

For statistical analysis, multiple comparisons among the four groups (SER, Zi, Tm and controls) were performed by a one-way ANOVA and Bonferroni's t test using the statistical package, BMDP 6D, 7D and 1V (Chasen, 1985; Dixon, 1985; Engelman, 1985) on an IBM main-frame computer system 4381. A linear regression analysis was performed to investigate the relationship between the axon perimeter and the number of myelin lamellae, and correlation coefficients were calculated to indicate the strength of any such linear relationship. Furthermore, we performed the test of H: $\varrho = 0$, where ϱ is the correlation coefficient. Analysis of covariance was used to test the equality of the coefficients for each covariate of the slopes of the regression lines between the controls and each mutant rat.

RESULTS

Gross and light microscopic observations

On macroscopic observation at 3 wks of age, the size of the brainstem and spinal cord of the three mutant rats, especially SER and Zi, was smaller than that for controls. However, there was no difference in cerebral size between any of these mutants and the control rats. In paraffin-embedded sections treated with the Klüver-Barrera stain, white matter pallor was observable, most prominantly in SER, then in Zi, and least in Tm rats. In addition, vacuolation in the thalamus, brainstem and cerebellar white matter occurred prominently in SER and in Tm. In epoxy resin sections stained with toluidine blue, hypomyelination in the CNS was apparent in SER and Zi and, to a lesser extent in Tm. The myelin sheath was disproportionately thin relative to axon calibre, and aberrant myelin sheaths were easily recognized, especially in SER and Zi rats (figs 1, 2). Glial cells in the white matter were also prominent in SER and Tm. Vacuolation was present mainly in the grey matter neuropil most prominantly in SER, less so in Tm, and least in Zi. The density of neurons seemed to be slightly decreased in the markedly vacuolated areas, the contours of the cytoplasm and the nuclei of the neurons

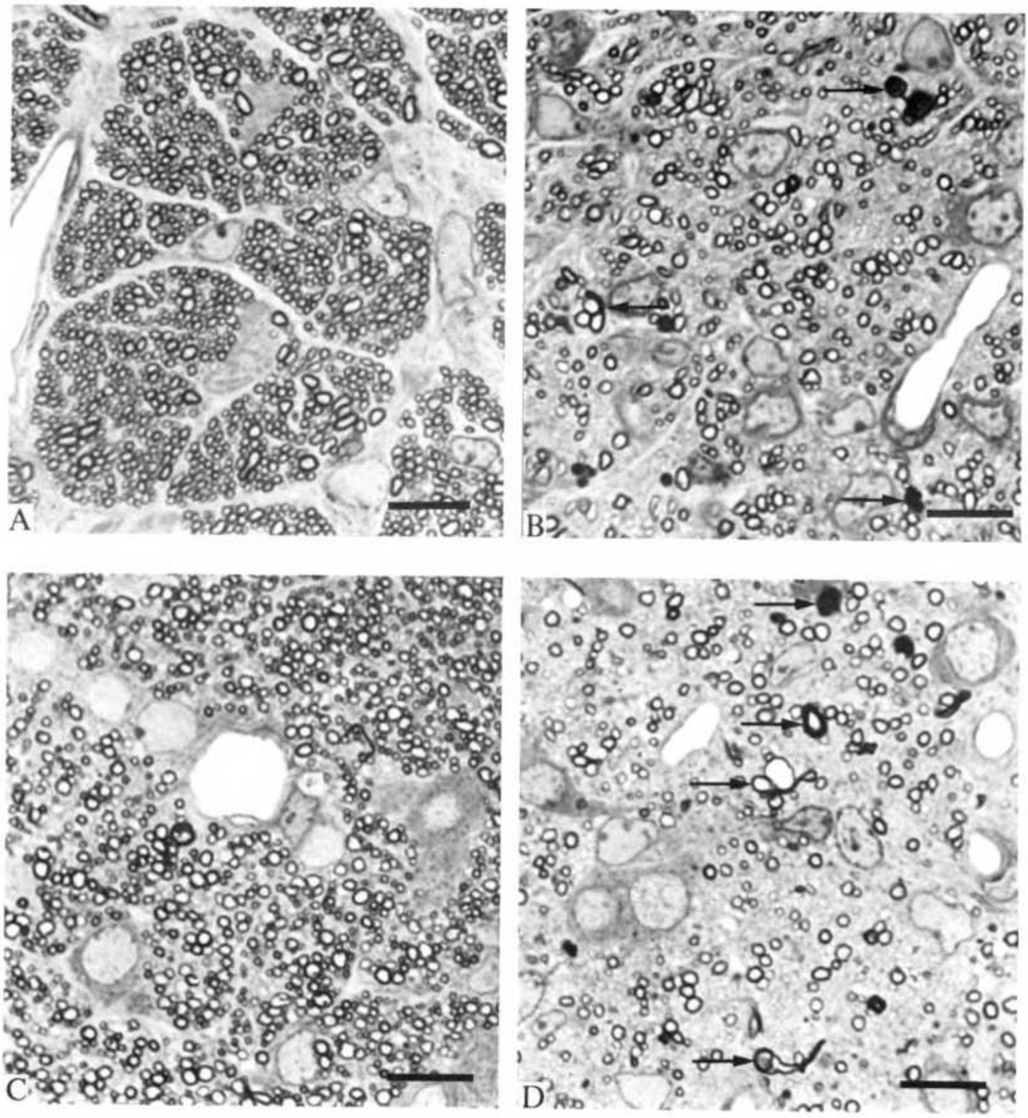

FIG. 1. Light micrographs of optic nerves at 3 wks of age. Epoxy resin embedded sections. Toluidine blue stain. A, *control rat*. Many small myelinated fibres are present with clustering. Bar = 10 μm. B, *Zi*. The number of myelinated fibres is far fewer than that of controls and there is no tendency to clustering. Arrows indicate the aberrant myelin sheaths. Bar = 10 μm. C, *Tm*. The number of myelinated fibres is moderately decreased from that of controls and glial cells are prominent. No clustering is observed. Bar = 10 μm. D, *SER*. Myelinated fibres are decreased in number. Aberrant myelin sheaths (arrows) are most prominent in these mutant rats. Bar = 10 μm.

appeared to be normal, and there were no degenerative changes or accumulations debris. In addition, there were no inflammatory cells or lipid-laden macrophages (fig.3).

At 12 wks of age, ventricular dilatation had developed in SER and Tm rats, while the size of the spinal cord in SER and Zi was still smaller than in the controls. Myelin

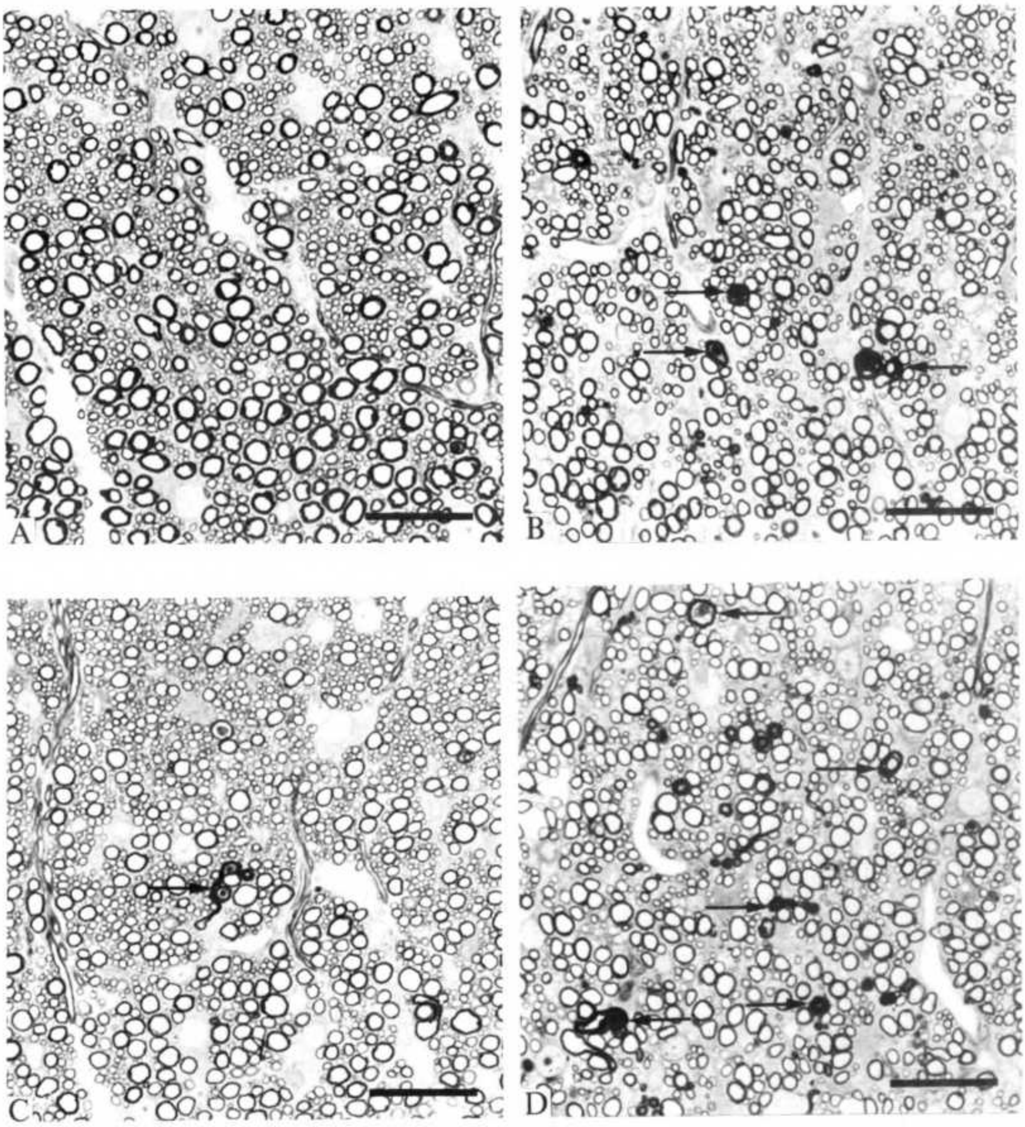

FIG. 2. Light micrographs of anterior column of the cervical spinal cord at 3 wks of age. Epoxy resin embedded sections. Toluidine blue stain. A, *control rat*. Bar = 30 μm. B, *Zi*. Hypomyelination with redundant and aberrant myelin sheaths (arrows). Bar = 30 μm. C, *Tm*. Hypomyelination is present, but aberrant myelin sheaths are rare (an arrow indicates one typical aberrant myelin sheath). Bar = 30 μm. D, *SER*. Both hypomyelination and aberrant myelin sheaths (arrows) are prominent. Bar = 30 μm.

pallor with the Klüver-Barrera stain was still distinct throughout the CNS of these 3 mutant rats, especially SER and Zi. Vacuolation in the thalamus and brainstem was readily seen in Zi but was definitely milder than that in SER and Tm. The degree and distribution of vacuolation is summarized in Table 2. In SER and Zi, both axon calibre and the thickness of the myelin sheath were retarded in their development. Although

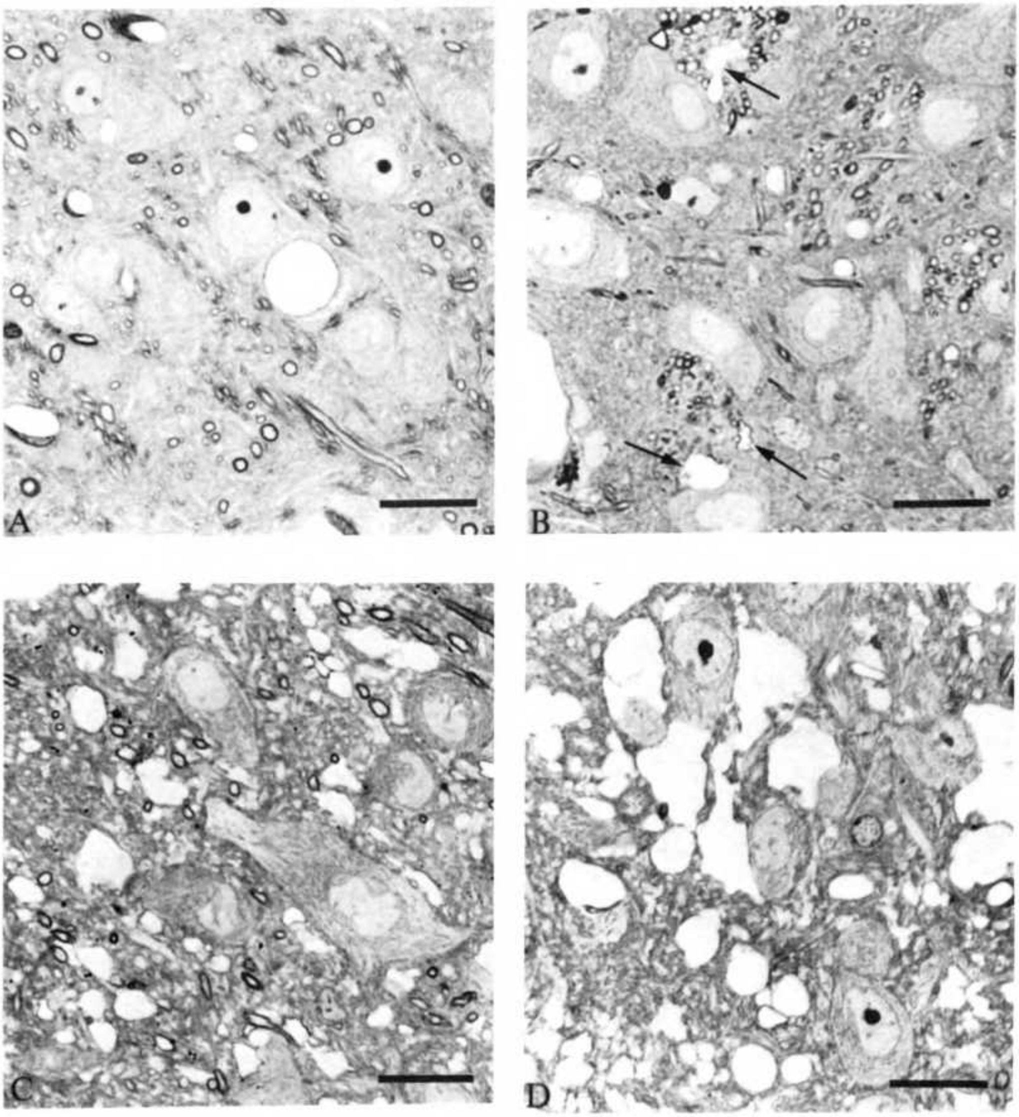

FIG. 3. Light micrographs of the thalamus at 3 wks of age. Epoxy resin embedded sections. Toluidine blue stain. A, *control rat.* Bar = 20 μm. B, *Zi*. A few small vacuoles are observed (arrows). The density and structure of neurons appear to be normal. Bar = 20 μm. C, *Tm*. Many vacuoles are observed but their size is mostly smaller than that of neurons. The density and structure of neurons appear to be normal although irregularity of the nuclear membrane is prominent in this field. Bar = 20 μm. D, *SER*. Vacuoles tend to be fused and the size of some large vacuoles is similar to that of neurons. Bar = 20 μm.

axon calibre grew in size in Tm similarly to the controls, the myelin sheaths were still slightly thinner in Tm. Aberrant myelin sheaths slightly decreased in number at this age. However, they still remained a prominent feature in SER. Occasionally, periaxonal space enlargement was observed in SER and Tm, especially in the optic nerves.

TABLE 2 DEGREE AND DISTRIBUTION OF VACUOLATION

		Location				
Rat	*Age (wks)*	*Cerebrum*	*Thalamus*	*Pons*	*Cerebellum*	*Spinal cord*
Zi	3	−	±	+	±	±
	12	±	+	+	+	+
	30−34	+	+	++	+	+
	37	+	+	++	+	+
Tm	3	+	++	+++	+	+
	12	++	++	+++	++	+
	47	++	+++	+++	++	+
SER	3	+	+++	+++	++	++
	12	++	+++	+++	+++	++

− = absent; ± = very mild, + = mild; ++ = moderate; +++ = severe The degree of vacuolation was determined from light microscopic observations on epoxy resin-embedded semithin sections of all mutant rats at each age. Absent = no vacuoles in high power magnified field (HPF) approximately ×200, very mild = a few small vacuoles in HPF, mild = small vacuoles scattered almost consistently in every HPF, moderate = vacuoles consistently observed but size of vacuoles mostly smaller than that of neurons; severe = some vacuoles fused to each other and of larger size than neurons.

Zi developed ventricular dilatation after 30 wks of age, with a persistently smaller size of the spinal cord. Aberrant myelin sheaths again became prominent findings in Zi after this age.

In Tm at 47 wks of age, the main pathological findings were ventricular dilatation, marked vacuolation and pseudocalcification in the thalamus. However, the degree of hypomyelination was no greater than at 12 wks of age.

Throughout the period of observation, cortical and subcortical neurons mostly maintained a normal appearance, although the cytoplasm was occasionally compressed by adjacent large vacuoles. The peripheral nervous system (PNS), including trigeminal nerves, dorsal root ganglia, spinal roots, and the sciatic nerves of these 3 mutant rats appeared normal, and there was no difference between any of these mutants and the controls.

Electron microscope observations

The ultrastructural alterations of hypomyelination and vacuolation were essentially similar in Zi, Tm and SER, although their severity, chronology and distribution were slightly different in each kind of mutant rat.

Some myelinated axons and many unmyelinated axons were present around single oligodendrocytes which revealed a normal nuclear contour and a tiny perikaryon. There were neither degenerative changes nor structural abnormalities in the organelles of the oligodendrocytes (fig. 4). Myelin sheath structure appeared normal and the periodicity of the major dense line and the interperiod line was similar to that of controls. However, the number of myelin lamellae in these mutant rats was fewer than that in controls. Elongated redundant myelin sheaths with or without a small axon were frequently observed (fig. 5A). Aberrant myelin sheaths did not enclose axons in many instances and showed a loose scroll-like structure (fig. 5B). Myelin lamellae split from the inner loop were occasionally seen.

Vacuoles related to expansion of extracellular spaces, in astrocytic processes without

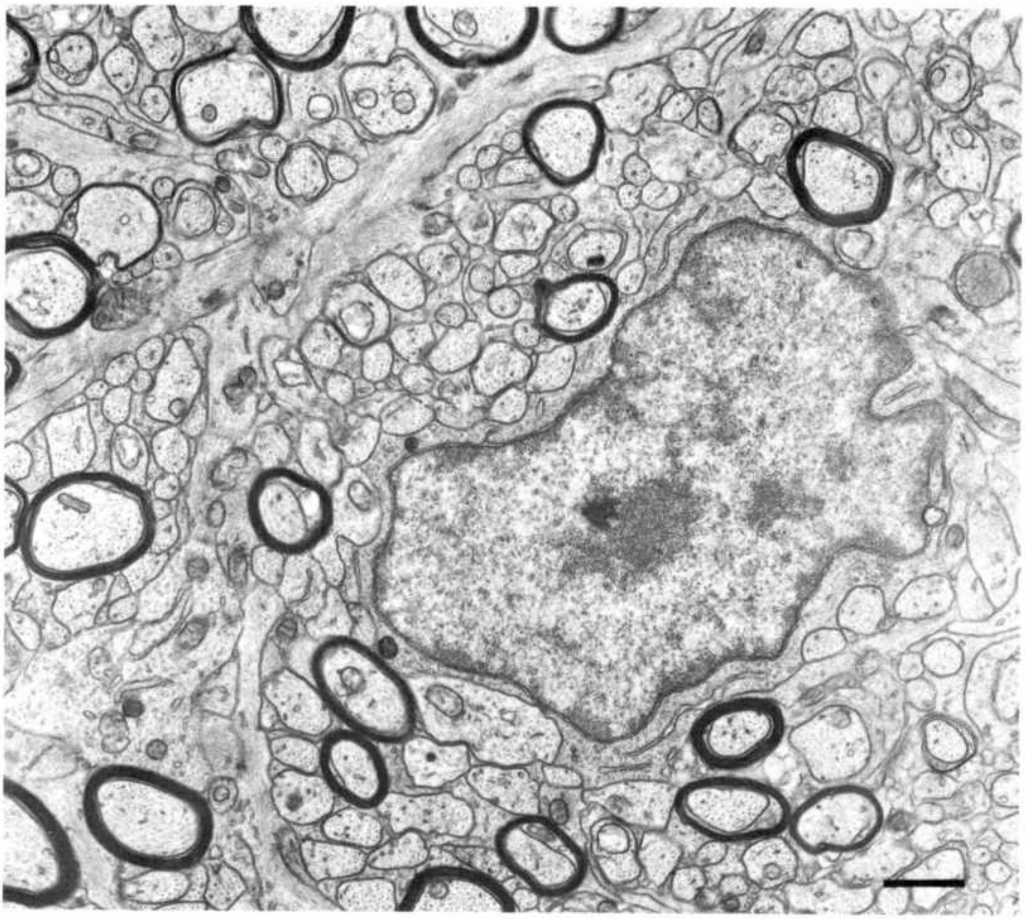

FIG. 4. Electron micrograph of an oligodendrocyte in the optic nerve of a 3 wk *Zi* rat. One oligodendrocyte with a slightly irregular nuclear contour and scanty cytoplasm has a few axons in the surrounding area which should have been covered by its myelin-forming processes. Bar = 1 μm.

cellular alterations, or in expanded periaxonal spaces without accompanying axonal degeneration were found especially in Tm. In areas of advanced degenerative change, astrocytic processes around the blood vessels frequently were swollen and were electron-lucent. However, the basal lamina around vessels appeared to be intact, tight junctions were not disrupted and endothelial pinocytotic vesicles were not increased in number.

The ultrastructure of the neurons appeared to be normal. There was no increase of lysosomes or lipofuscin in their cytoplasm. As a rare finding, small cytoplasmic vacuoles and crenation of the cell membrane were seen adjacent to vacuoles (fig. 6).

The radial component of myelin (Honjin, 1959; Honjin *et al.*, 1963) that had an electron-lucent linear structure (an electron-dense linear structure when the material was fixed or stained with potassium permanganate) across the myelin lamellae was prominent in all three types of mutant rat. In comparison with control rats, the number of radial components per myelinated axon appeared to be increased (in optic nerves

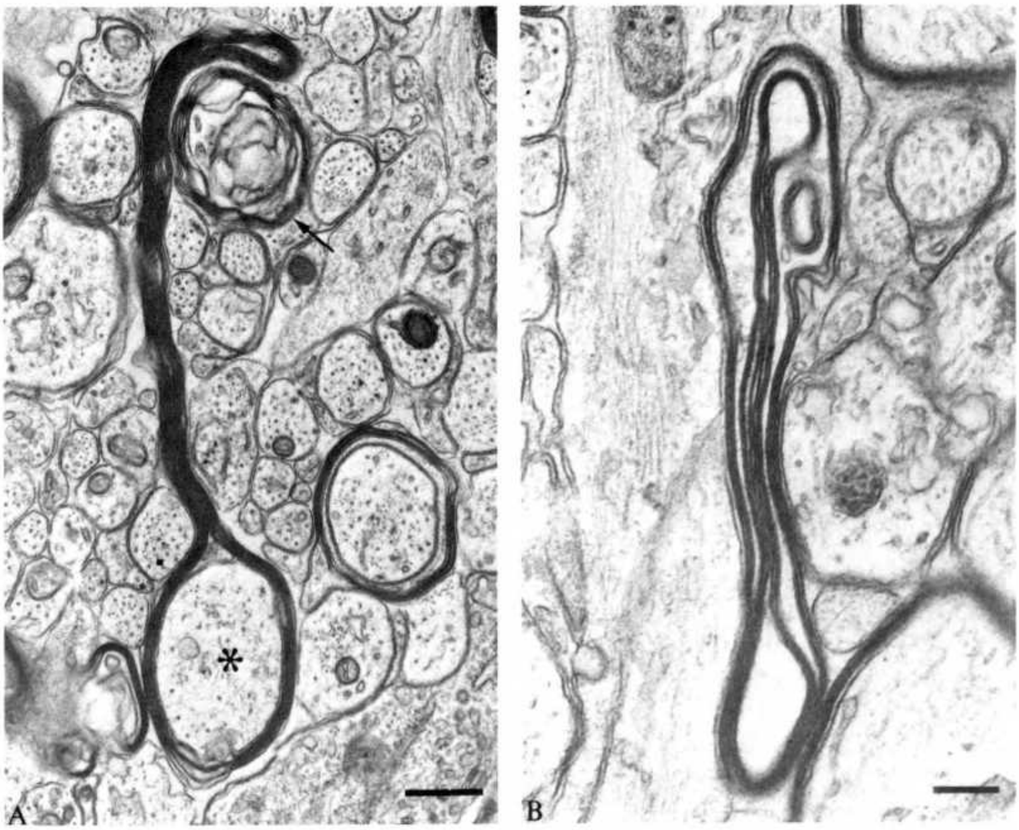

FIG. 5. Electron micrographs of abnormal myelin sheaths. A, optic nerve of 3 wk *SER* rat. An elongated redundant myelin sheath encloses a cytoplasmic process, probably axonal (*), at one end. A scroll-like myelin figure is also seen (arrow). Bar = 0.5 μm. B, optic nerve of 3 wk *Zi* rat. An aberrant flap of myelin consisting of a few lamellae forms a loose scroll-like structure. Bar = 0.2 μm.

7.1 ± 3.6 in SER, 7.8 ± 3.6 in Zi and 7.5 ± 2.6 in Tm, as compared with 2.8 ± 0.6 in control rats, at 3 wks of age, each value given as mean ± SD) (fig. 7A). Occasionally, radial components appeared not only between the inner and outer loops originating from one oligodendrocyte process but also between cytoplasmic islets of oligodendrocytes in the myelin lamellae (fig. 7B).

Some astrocytes showed abundant cytoplasm containing many bundles of glial filaments and lysosomes in all three types of mutant rat, even at 3 wks of age (fig. 8).

In older Zi and Tm, degenerative alterations in the axons were sometimes observed, surrounded by an aberrant myelin sheath. The extracellular space was enlarged, causing the cell processes or axons with or without myelin sheath to be separated.

There was no evidence of cellular phagocytic activity in any of the material.

Quantitative analysis for hypomyelination

Anterior columns of spinal cord at C1 – C2 level. Myelinated fibre density in SER was markedly decreased, and aberrant myelin sheaths in the mutant rats, especially in

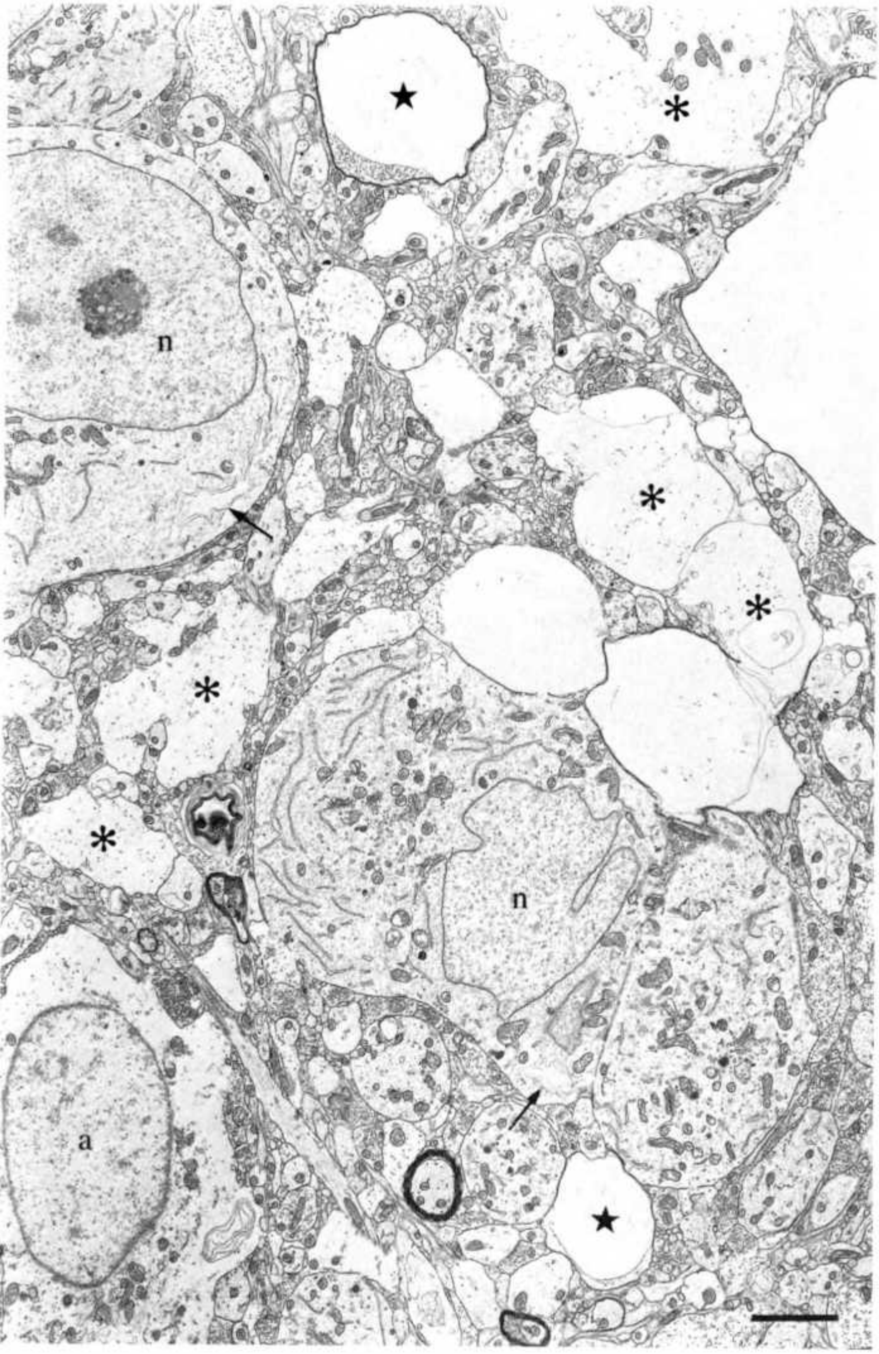

FIG. 6. An electron micrograph of the thalamus of 3 wk *Tm* rat. The structure of 2 neurons (n) appears to be normal, except for small vacuoles in the cytoplasm (arrows). The cytoplasm of an astrocyte (a) is electron-lucent and astrocytic processes are swollen and electron-lucent (∗). Some vacuoles surrounded by a few myelin lamellae presumably originate from oligodendrocytic processes (★). No degenerative material or increased numbers of lysosomes are seen in neurons. Bar = 3 μm.

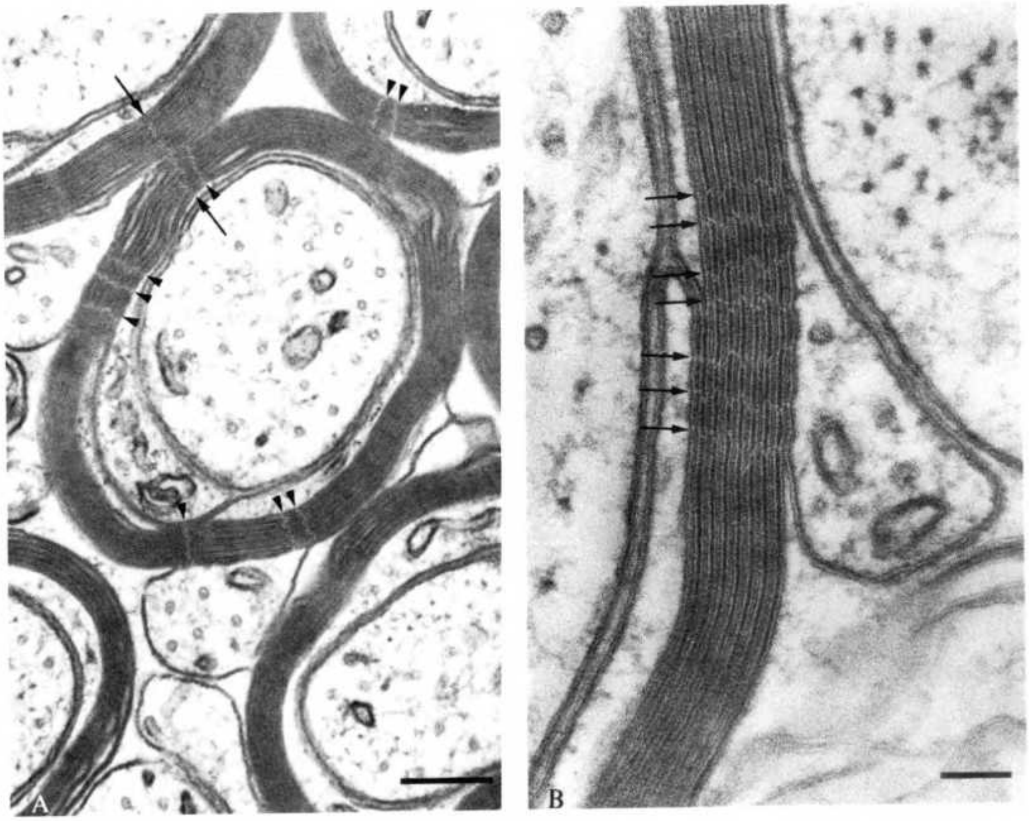

FIG. 7. Electron micrographs of radial component of myelin. A, *Tm* optic nerve at 3 wks of age. Radial components (arrow heads) extend through from the inner to the outer lamellae of myelin sheaths. One radial component connects directly to an adjoining myelin sheath (arrows). Bar = 0.2 μm. B, *SER* optic nerve at 3 wks of age. The structure of the radial components and myelin lamellae themselves is normal but the number of radial components is increased (arrows indicate each of them). Bar = 0.1 μm.

SER, were more numerous at 3 wks of age, as compared with controls. The density of glial cells—astrocytes and oligodendrocytes—was also increased in SER and Tm (Table 3). The maximum number of myelin lamellae in the mutant rats was disproportionally less than in controls (fig. 9), being less than 40 in Zi and Tm, and less than 30 in SER. In addition, all axon perimeters in SER were less than 12 μm.

At 12 wks of age, myelinated fibre density in SER had become almost the same as that in the controls, while in Zi it had slightly decreased. Aberrant myelin sheaths were most evident in SER and less so in Zi and Tm, in that order (Table 3). The density of glial cells was increased in Tm and SER. The regression lines between axon perimeter and the number of myelin lamellae of Zi and Tm were steeper than at 3 wks, which may indicate a recovery from the developmental delay of myelination. However, that for SER remained the same as at 3 wks of age (fig. 10).

The slope of the regression line in Zi rats older than 12 wks began to decline again with age (data from 37 wks are shown in Table 3 and fig. 11). Tm at 47 wks of age

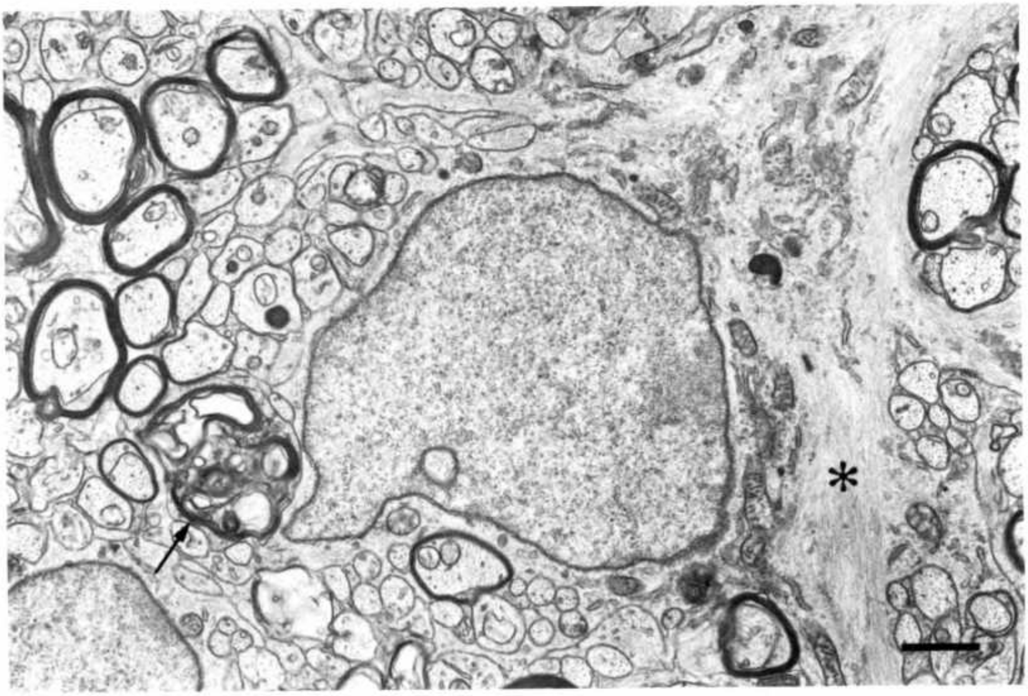

FIG. 8. An electron micrograph of an astrocyte of a *Zi* rat at 3 wks of age. One astrocyte has prominent cytoplasm with abundant glial filaments (*). An aberrant or degenerating myelin sheath is also seen (arrow). Bar = 1 μm.

did not reveal any definite hypomyelination and the regression line of Tm ($y = 4.45x+9.61$, $n = 69$, $r = 0.88$, $P < 0.001$) was not significantly different from that of controls ($y = 5.25x+5.48$, $n = 70$, $r = 0.91$, $P < 0.001$).

Optic nerve. The transverse sections of the optic nerve in SER were slightly but not significantly larger than those from Zi, Tm or control rats at 3 wks of age. However, the number of myelinated fibres in SER was almost one-third that of controls. The number of myelinated fibres in Zi and Tm was also about one-third and two-thirds of that in controls, respectively (Table 4). The aberrant myelin sheaths in SER and Zi were markedly increased in number. Glial cells appeared to be more prominent in SER (Table 4). The correlations of the axon perimeter and the number of myelin lamellae between any of these mutant rats and the controls showed definite hypomyelination in mutant rats, especially Zi, at this age. The regression lines were as follows: controls: $y = 2.30x+4.22$, $n = 260$, $r = 0.61$, $P < 0.001$; Zi: $y = 0.90x+3.57$, $n = 83$, $r = 0.33$, $P = 0.002$; Tm: $y = 1.93x+3.72$, $n = 187$, $r = 0.66$, $P < 0.001$; SER: $y = 1.84x+1.92$, $n = 67$, $r = 0.51$, $P < 0.001$. The slope of the regression line for Zi was significantly less than that of the controls ($P < 0.01$) (Table 4).

At 12 wks of age, the transverse sections of the optic nerves of Zi were smaller in size than those of Tm or of controls. Nevertheless, all optic nerves developed in size after 3 wks of age. Although the aberrant myelin sheaths in SER were still frequent,

TABLE 3. QUANTITATIVE DATA OF HYPOMYELINATION IN SPINAL CORD

	Density of myelinated fibres (/mm^2)	*Density of aberrant myelin sheaths (/mm^2)*	*Density of glial cells (/mm^2)*
3 wks			
Control (n = 3)	154295 ± 1456	0	459 ± 29
Zi (n = 3)	128313 ± 4075★☆	270 ± 15★☆	559 ± 50
Tm (n = 3)	175923 ± 2934★★	87 ± 7★★	845 ± 75★★★
SER (n = 3)	94944 ± 1310★☆	498 ± 11★☆	802 ± 87★★★
12 wks			
Control (n = 3)	101673 ± 3008	0	224 ± 17
Zi (n = 3)	80465 ± 4205★★★	210 ± 13★☆☆	316 ± 15
Tm (n = 3)	96551 ± 2550	48 ± 15	383 ± 18★★
SER (n = 3)	97650 ± 3904	435 ± 35★☆	360 ± 25★★
37 wks			
Control (n = 3)	93985 ± 1059	8 ± 1*	194 ± 16
Zi (n = 3)	61865 ± 2755★	299 ± 40★★	221 ± 14
47 wks			
Control (n = 3)	89197 ± 481	9 ± 3*	182 ± 8
Tm (n = 3)	86827 ± 903	55 ± 12★★★	256 ± 25

n = no. of animals. Values are mean ± SEM. ★ $P < 0.001$, compared with control. ☆ $P < 0.001$, compared with Tm. ★★ $P < 0\,01$, compared with control ☆☆ $P < 0.01$, compared with Tm. ★★★ $P < 0.05$, compared with control. * The value includes age-related myelin degeneration.

there was no significant difference in the numbers of glial cells between all 3 mutant rats and the controls. However, hypomyelination persisted in the mutant rats as compared with the controls. The regression lines were as follows: control: $y = 3.19x+1.00$, $n = 226$, $r = 0.76$, $P < 0.001$; Zi: $y = 2.51x+0.28$, $n = 133$, $r = 0.72$, $P < 0.001$; Tm: $y = 2.70x+1.04$, $n = 189$, $r = 0.87$, $P < 0.001$; SER: $y = 2.37x+2.06$, $n = 127$,$r = 0.70$, $P < 0.001$. The slope of the regression line for SER was significantly less than that for the controls ($P < 0.01$).

Zi older than 12 wks continued to show a smaller number of myelinated fibres and a progressively increased number of aberrant myelin sheaths in comparison with the controls (data at 37 wks are shown in Table 3). The regression line again declined from that at 12 wks. The regression lines were as follows: controls: $y = 3.33x+1.21$, $n = 129$, $r = 0.83$, $P < 0.001$; Zi: $y = 1.57x+2.53$, $n = 99$, $r = 0.55$, $P < 0.001$. The regression line for Zi was significantly less than that of the controls ($P < 0.01$).

Tm at 47 wks showed the mildest degree of hypomyelination. The regression lines of Tm ($y = 3.80x+1.72$, $n = 63$, $r = 0.91$, $P < 0.001$) and of controls ($y = 4.01x+1.90$, $n = 118$, $r = 0.84$, $P < 0.001$) showed almost the same slope. There was no significant difference in the slopes of the regression lines between Tm and controls. Aberrant myelin sheaths in Tm were rare, although the number of myelinated fibres in the optic nerve was decreased in comparison with controls.

Sciatic nerve. Throughout the observations, there was no significant difference among the three types of mutant and the controls, both in numbers of myelinated fibres (Table 5) and the regression lines between axon perimeter and the number of myelin lamellae

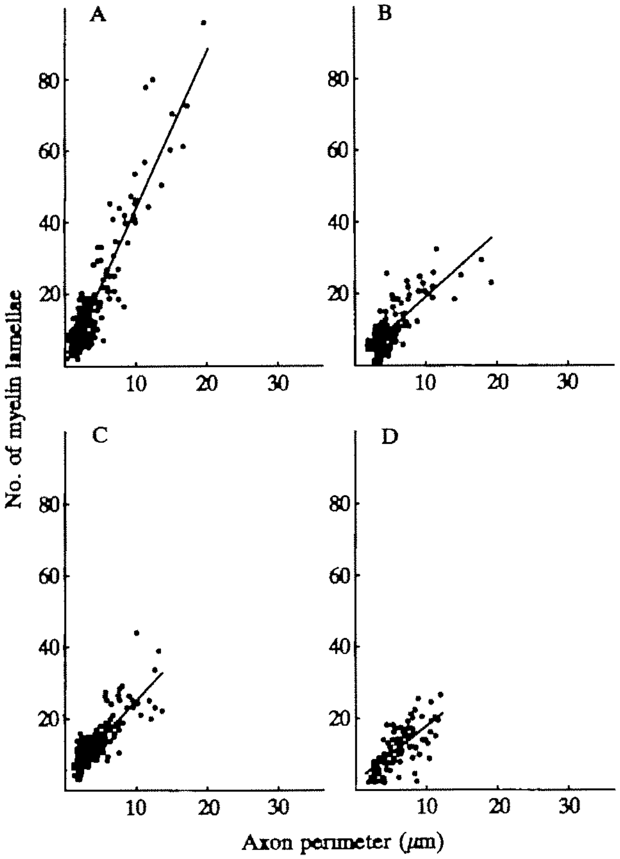

FIG. 9. Correlation between axon perimeter and number of myelin lamellae in the anterior columns of the cervical spinal cord at 3 wks of age A, control rat. Regression line: $y = 4.40x-0.63$, $n = 148$, $r = 0.92$, $P < 0.001$. B, *Zi* Regression line: $y = 1.70x+2.20$, $n = 133$, $r = 0.77$, $P < 0.001$. C, *Tm* Regression line: $y = 2.01x+4.34$, $n = 173$, $r = 0.83$, $P < 0.001$. D, *SER*. Regression line: $y = 1.59x+1.78$, $n = 90$, $r = 0.67$, $P < 0.001$ The slopes of the regression lines of Zi, Tm and SER are significantly less than that of the controls ($P < 0.01$).

(e.g., controls: $y = 5.99x+4.91$, $n = 75$, $r = 0.92$, $P < 0.001$, and SER: $y = 6.18x+3.48$, $n = 71$, $r = 0.95$, $P < 0.001$, at 12 wks of age).

DISCUSSION

The results described here clearly demonstrate the presence of hypomyelination in the CNS in these three mutant rats, especially in SER and Zi, in addition to the presence of vacuolation. Because the phenotypes of Zi and Tm are rather similar, allelism had been suspected. However, the segregation data of subsequent crosses between Zi and Tm indicated that *zi* and *tm* are located on different chromosomes (Serikawa and Yamada, 1986). The *zi* locus is assigned to linkage group IV (Yamada *et al.*, 1989), although linkage analysis for *tm* has not yet been reported (preliminary analysis has suggested that *tm* relates to linkage group II). Each mutation must be correlated with the clinical feature and the neuropathology even if these are not specific to one mutation. It is also

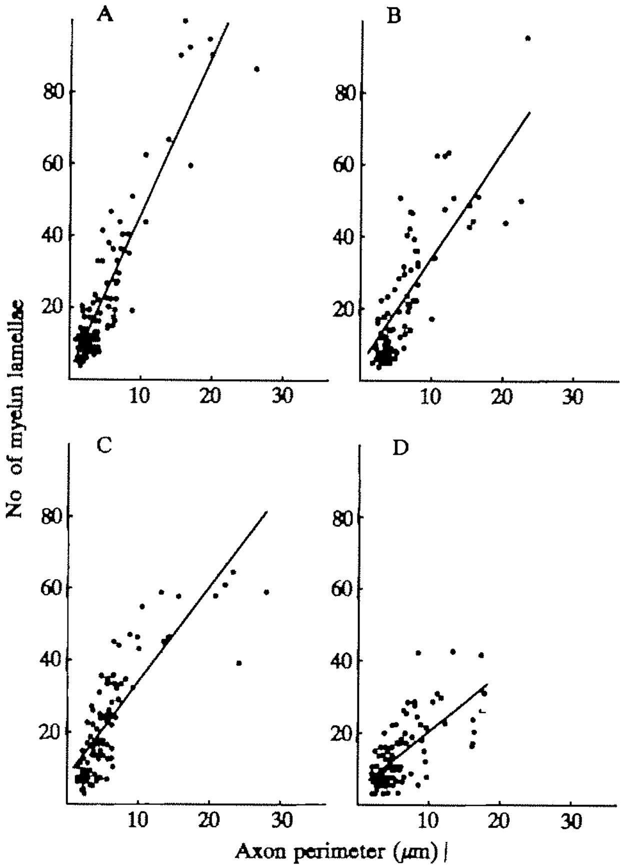

FIG 10. Correlation between axon perimeter and number of myelin lamellae in the anterior columns of the cervical spinal cord at 12 wks of age A, control rat. Regression line: $y = 4.39x+2.81$, $n = 99$, $r = 0.92$, $P < 0.001$. B, *Zi*. Regression line $y = 3.02x+4.14$, $n = 85$, $r = 0.75$, $P < 0.001$. C, *Tm* Regression line $y = 2.51x+7.76$, $n = 97$, $r = 0.83$, $P < 0.001$. D, *SER* Regression line. $y = 1.60x+4.73$, $n = 101$, $r = 0.66$, $P < 0.001$ The slopes of the regression lines of Zi, Tm and SER are significantly less than that of the controls ($P < 0.01$).

important to determine how each genetic abnormality interacts and produces the clinical and pathological alterations in the double mutant SER.

As the degree of hypomyelination in SER and Zi was more severe than that in Tm, the gene responsible for hypomyelination may be derived from the zitter mutation. It is postulated that hypomyelination might be due to developmental delay and the functional abnormality in oligodendrocytes, because morphological examination did not reveal any structural abnormality in the nucleus or perikarya of the oligodendrocyte or in the myelin sheath in these mutant rats, throughout CNS development.

In chronological observations investigating the correlation between the axon perimeter and the number of myelin lamellae in the control rats, the slopes of the regression lines for the cervical spinal cord remained almost constant. However, those of the optic nerves gradually steepened with advancing age. Meanwhile, the total number of myelinated fibres increased to a maximum at 12 wks of age, then gradually decreased thereafter.

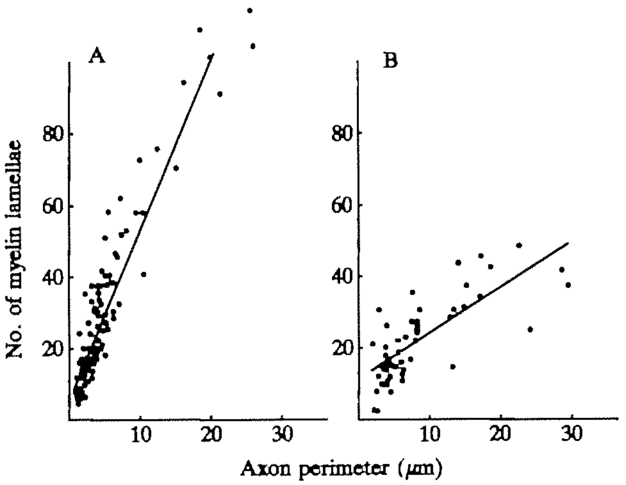

FIG. 11. Correlation between axon perimeter and number of myelin lamellae in the anterior columns of the cervical spinal cord at 37 wks of age. A, control rat. Regression line $y = 4.41x+6.95$, $n = 92$, $r = 0\ 94$, $P < 0.001$. B, Zi. Regression line: $y = 1.28x+6.6$, $n = 54$, $r = 0\ 77$, $P < 0\ 001$. The slope of the regression line of Zi is significantly less than that of the controls ($P < 0\ 01$).

TABLE 4 QUANTITATIVE DATA ON HYPOMYELINATION IN OPTIC NERVE

	Nerve area (mm^2)	*No. of myelinated fibres*	*No of aberrant myelin sheaths*	*No. of glial cells*
3 wks				
Control (n = 3)	0.103±0.007	59887±557	0	261±2
Zi (n = 3)	0.106±0.013	21588±1717★☆	70±5★	239±23
Tm (n = 3)	0.100±0 009	41603±1281★	17±1	243±7
SER (n = 3)	0 138±0 008	20141±969★☆	84±8★☆	385±4★☆
12 wks				
Control (n = 3)	0.232±0.010	95814±1773	1.6±0.9	227±14
Zi (n = 3)	0.138±0 003★★☆☆☆	34776±1778★☆	19±4	229±9
Tm (n = 3)	0.193±0.014	73804±4323★★	10±2	251±20
SER (n = 3)	0 168±0 013	42040±1183★☆	54±6★☆	261±6
37 wks				
Control (n = 3)	0.279±0.040	71620±1271	2 3±0.3*	186±12
Zi (n = 3)	0.211±0.010★★	41506±1023★	39±4★★	223±5★★★
47 wks				
Control (n = 3)	0.259±0.013	67079±1888	4±1*	185±4
Tm (n = 3)	0.268±0.002	47254±2624★★	25±4★★	197±9

n = no. of animals Values are mean ± SEM. ★ $P < 0.001$, compared with control. ☆ $P < 0.001$, compared with Tm. ★★ $P < 0\ 01$, compared with control. ☆☆☆ $P < 0\ 05$, compared with Tm. ★★★ $P < 0.05$, compared with control. * The value includes age-related myelin degeneration.

Finally, the values correlated well with the numbers reported previously (Bruesch and Arey, 1942). These facts are thought to indicate that the developmental process in the optic nerve occurs later than that of the spinal cord, even in control rats. The sequential changes in the regression lines seem to indicate a developmental delay in myelination

TABLE 5 QUANTITATIVE DATA ON MYELINATED FIBRES IN SCIATIC NERVE

	Nerve area (*mm*^{2})	*No. of myelinated fibres*	*No. of Schwann cells*
3 wks			
Control (n = 3)	0.190 ± 0.007	7155 ± 337	582 ± 23
Zi (n = 3)	0.190 ± 0.004	7204 ± 369	581 ± 30
Tm (n = 3)	0.189 ± 0.004	6854 ± 136	573 ± 35
SER (n = 3)	0.189 ± 0.008	7188 ± 114	565 ± 11
12 wks			
Control (n = 3)	0.545 ± 0.054	8224 ± 29	437 ± 37
Zi (n = 3)	0.433 ± 0.015	8109 ± 117	463 ± 27
Tm (n = 3)	0.472 ± 0.028	8240 ± 445	452 ± 43
SER (n = 3)	0.469 ± 0.033	8650 ± 487	451 ± 40
37 wks			
Control (n = 3)	0.666 ± 0.016	8272 ± 769	485 ± 39
Zi (n = 3)	0.661 ± 0.015	7853 ± 129	504 ± 23
47 wks			
Control (n = 3)	0.711 ± 0.064	8827 ± 590	501 ± 38
Tm (n = 3)	0.655 ± 0.004	8166 ± 421	459 ± 36

n = no. of animals. Values are means ± SEM

in Zi and Tm. However, hypomyelination in Zi again progressed after 12 wks of age when the regression lines steepened and aberrant myelin sheaths transiently decreased. These chronological changes in hypomyelination correlated well with the clinical course of the tremor. At around 2 wks of age, tremor developed in these mutant rats, became most obvious between 3 and 5 wks of age then declined gradually in SER and Tm (Yamada *et al.*, 1985; Serikawa and Yamada, 1986), while it continued at the same high level in Zi (Rehm *et al.*, 1982).

Vacuolation in the CNS might be considered to be an aetiological factor for the development of epilepsy in these mutant rats because, in chronological observations, marked vacuolation in the CNS of SER and Tm were present even at 3 wks of age, and remained at almost the same severity or slightly increased with age. Clinically, Tm older than about 14 wks often manifested a petit mal type epilepsy (Serikawa *et al.*, 1987). SER developed spontaneous tonic convulsions and a petit mal type epilepsy at around 7 or 8 wks of age and they became critical at around 14 wks. All SER died before 20 wks (Serikawa and Yamada, 1986). Although mild to moderate vacuolation in the CNS also developed in Zi with advancing age, they manifested only flaccid paresis and not epilepsy (Rehm *et al.*, 1982). Thus epilepsy is thought to be derived from tremor mutation and is to some extent related to advancing vacuolation.

Many aetiological factors have been postulated as a cause of vacuolation. First, methodological artefacts must be excluded. However, it is thought to be the least likely because other fine structures such as the mitochondria and myelin lamellae were quite well preserved even in markedly vacuolated areas and the degree and distribution of vacuolation were constant in each mutant rat of the same age. Although the ultrastructure of the vacuoles in these mutant rats had some similarities to those in experimental animals with intoxication, for example, by cuprizone (Suzuki and Kikkawa, 1969), triethyl tin (Lee and Bakay, 1965), or isoniazid (Blakemore *et al.*, 1972), extrinsic factors seem improbable as a cause. Experimental ammonia encephalopathy induced by methionine sulfoximine provides a clue to neuropathological alteration in a condition manifested by seizures and high blood ammonia levels (Cole *et al.*, 1972; Gutierrez and Norenberg, 1977). As the pathology in this encephalopathy was mainly restricted to astrocytes, which showed accumulations of glycogen, it was different from that in the mutant rats in the present study; but it is postulated that these mutant rats share some similar pathological processes with such intoxicated animals.

In the double mutant rat, the two genetic abnormalities—tremor with hypomyelination from zitter mutation, and epilepsy with vacuolation from tremor mutation—may have interacted with each other. The clinical manifestations and pathological changes seemed to be accelerated, suggesting synergistic reinforcement. It also seems that the hypomyelination promoted the occurrence of epilepsy in SER, just as white matter lesions are sometimes closely related to the course of epilepsy in patients with multiple sclerosis and adrenoleucodystrophy (Kurtzke, 1970; Schaumburg *et al.*, 1975). To elucidate the pathological causes of tremor and epilepsy in these mutant rats, observations should be made on the pathological changes in the CNS following the reported clinical and electrophysiological improvement obtained by antiepileptic drugs (Sasa *et al.*, 1988).

It seems important to compare the hypomyelination in the CNS of these mutant rats with those of other animal models of hypomyelination, especially in rodents. Several varieties of mutant mice have been reported previously; the quaking mouse (Sidman *et al.*, 1964; Samorajski *et al.*, 1970; Wiśniewski and Morrell, 1971) showed hypomyelination both in the CNS and the PNS with autosomal recessive inheritance; jimpy (Sidman *et al.*, 1964; Hirano *et al.*, 1969; Meier and Bischoff, 1975) showed hypomyelination only in the CNS with X-linked inheritance; shiverer (Privat *et al.*, 1979) also showed CNS hypomyelination, and trembler (Falconer, 1951; Ayers and Anderson, 1973) showed only PNS hypomyelination. Twitcher (Duchen *et al.*, 1980), which is a murine model for globoid cell leucodystrophy, showed hypomyelination both in the CNS (Nagara *et al.*, 1982*a*) and PNS (Kondo *et al.*, 1988). Recently, a double mutation with jimpy and shiverer has been reported and has displayed some interesting combinations of pathological findings which are derived from each mutant inheritance (Billings-Gagliardi *et al.*, 1986, 1987). Grey tremor mutant mouse has also been reported (Sidman *et al.*, 1985; Kinney and Sidman, 1986) and has revealed a combination of hypomyelination both in the CNS and PNS (greater in the PNS) and spongiform encephalopathy. This mouse seems to resemble most closely our mutant rats. In contrast to mutant mice, so far, to our knowledge, there is no established rat model for hypomyelination.

Among these mutant mice, abnormal oligodendrocytes were known to occur in jimpy and twitcher mice, which had characteristic inclusions in their cytoplasm (Privat *et al.*, 1972; Meier and Bischoff, 1974; Duchen *et al.*, 1980) and the number of oligodendrocytes in jimpy mice was known to be decreased (Meier and Bischoff, 1975), whereas no structural abnormality was found in our mutant rats. Shiverer mouse is characterized by absence of the major dense line in myelin sheath (Privat *et al.*, 1979) and quaking mouse revealed redundant myelin sheaths in the oligodendrocytic processes with a marked increase in numbers in the radial components (Nagara and Suzuki, 1981*b*, 1982*b*). An increased radial component and hypomyelination in SER and Zi appeared to be shared, in part, with the mutation in quaking mice.

Aberrant myelin and elongated myelin sheaths have not been stressed in these mutant mice, except transiently in the quaking mouse at around 5 – 15 days of age (Nagara and Suzuki, 1981*a*), these findings with hypomyelination were characteristic in SER and Zi. As excessive formation of myelin sheaths, referred to as elongated myelin sheaths or redundant flaps of myelin, has been observed in normally developing mice and rats (Luse, 1956; Peters, 1968) and in normal adult toads (Rosenbluth, 1966), the presence of such elongated myelin sheaths in adult mutant rats also indicated a developmental delay in myelin formation.

Finally, the mutant rats, Zi, Tm and SER, may be useful animal models for hypomyelination and vacuolation in the CNS which result in tremor and epileptic manifestations. The results reported here, particularly for SER, should reinforce the concept that two phenotypes derived from independent mutant genes, *zi/zi* and *tm/tm* reinforce each other clinically and neuropathologically.

ACKNOWLEDGEMENTS

The authors thank K. Hatanaka for histological preparations, M. Yoneda for photographic assistance, and the Koga Hospital for technical assistance. This research was supported in part by a Grant-in-Aid from the Ministry of Education, Science and Culture, Japan, and by a Grant-in-Aid from the Research Committee of Experimental Models for Intractable Diseases from the Ministry of Health and Welfare, Japan.

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(*Received November 20, 1989. Revised May 22, 1990. Accepted June 26, 1990*)

Brain (1991), **114**, 1001–1011

STIMULATION OF THE SUPERIOR SAGITTAL SINUS INCREASES METABOLIC ACTIVITY AND BLOOD FLOW IN CERTAIN REGIONS OF THE BRAINSTEM AND UPPER CERVICAL SPINAL CORD OF THE CAT

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SUMMARY

To investigate the anatomical distribution of cells concerned with relaying craniovascular nociception, local cerebral blood flow was examined in the cat using the tracer [^{14}C]-iodoantipyrine and local cerebral glucose utilization determined by the 2-deoxyglucose method in tissue autoradiographs. The superior sagittal sinus was stimulated electrically and its effect on caudal brainstem and upper cervical spinal cord blood flow and metabolism evaluated. This caused increased metabolic activity and blood flow in the trigeminal nucleus caudalis, in the cervical dorsal horn and in a discrete area in the dorsolateral spinal cord at the second cervical segment. Responses in these 3 areas were blocked by ablation of the trigeminal ganglia. The dorsolateral area activated in the spinal cord corresponds to a group of cells in or near the lateral cervical nucleus that may form an important relay for craniovascular nociception and thus be of relevance to the mechanism of headache.

INTRODUCTION

Migraine and other 'vascular' headaches involve pain that is mediated by the trigeminal and cervical sensory systems (Wolff, 1963; Lance, 1982). Intracranial vascular structures, such as the cerebral, meningeal and dural arteries and the dural sinuses, are innervated by the trigeminal nerve and have been thought to play an important role in the genesis of headache since Ray and Wolff (1940) demonstrated that electrical stimulation or distension of the cranial vessels can reproduce the site and quality of migrainous pain. Moreover, it is clear that both vascular and neural elements are important in migraine since changes in cerebral blood flow (Olesen *et al.*, 1981) and cortical function (Lashley, 1941; Wolff, 1963) are clearly documented.

Pain impulses from intracranial vascular structures traverse all three divisions of the trigeminal nerve although branches of the ophthalmic division, such as the tentorial nerve, primarily innervate supratentorial vessels (McNaughton, 1966). The tentorial nerves supply the superior sagittal sinus (SSS), a pain sensitive structure (Feindel *et al.*, 1960), while fibres from the trigeminal ganglion innervate branches of the middle cerebral artery (Mayberg *et al.*, 1984). Recent tracer studies using a horseradish peroxidase-wheat

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germ agglutinin conjugate applied to the middle cerebral artery of the rat (Arbab *et al.*, 1988) and electrophysiological observations from our laboratory (Lambert *et al.*, 1986) have suggested that, in addition to the conventional synapse of the first order trigeminal neurons in the spinal nucleus caudalis, cells in the second cervical segment of the spinal cord are involved in processing trigeminovascular afferents.

Field potential and single unit recordings of the trigeminal spinal nucleus (especially caudalis) and high cervical cord of the cat have demonstrated a discrete group of cells at the second cervical level of the spinal cord that consistently respond to electrical or chemical stimulation of the sagittal sinus and middle meningeal artery (Lambert *et al.*, 1986). The electrophysiological characteristics of these cells suggest that they receive A-delta and C fibre input and that they project to the thalamus (Zagami and Lambert, 1990). Indeed, cells responding to vascular stimuli are more readily identified at this site than in the more traditional nucleus caudalis (Lambert *et al.*, 1979).

In addition, SSS stimulation is a more powerful initiator of changes in regional cerebral blood flow than is stimulation of the trigeminal ganglion. Previous experiments have characterized increases in regional cerebral blood flow in the frontal and parietal cortex of the cat with trigeminal ganglion stimulation that are neurally mediated (Goadsby and Duckworth, 1987) but quantitatively less marked than changes elicited by SSS stimulation (Lambert *et al.*, 1988). In the latter situation, thalamic blood flow is increased whereas it is not altered by trigeminal ganglion stimulation. Similarly, SSS activation consistently leads to greater increases in evoked potentials in the dorsolateral C2 spinal cord than does trigeminal ganglion stimulation (Lambert *et al.*, 1988).

These experiments sought to determine what areas in the caudal brainstem and upper cervical spinal cord of the cat are activated when a pain sensitive to vascular structure, such as the SSS, is stimulated. We have attempted to determine whether craniovascular nociception is limited exclusively to known trigeminal relays or whether other structures are involved. Local glucose utilization and blood flow were used as indicators of neuronal activation and evaluated by autoradiographic techniques.

METHODS

Adult male or female cats were anaesthetized with alpha-chloralose (60 mg/kg), intubated, placed in a stereotaxic frame and paralysed with gallamine triethodide (6 mg/kg; Flaxedil, May and Baker). The animals were artificially ventilated via an endotracheal tube with a mixture of room air and oxygen. The end-expiratory CO_2 and inspiratory O_2 were continually monitored (DATEX Normocap, Helsinki, Finland) and the former adjusted by altering the tidal volume of the ventilator (Harvard Instruments, Harvard, MA, USA). Polyethylene catheters were inserted into the femoral artery and vein bilaterally for monitoring blood pressure, drug administration and blood sampling. Core temperature was continually monitored with a rectal thermistor and kept constant with a heating blanket. The level of anaesthesia was monitored by observing the animal's cardiovascular parameters during painful stimulation.

Surgery and stimulation

This preparation has been described in detail previously (Lambert *et al.*, 1988). Briefly, a midline craniotomy was performed and the bone carefully removed. Two parallel longitudinal incisions were made in the dura on either side of the sinus and then, after lifting the dural flaps, a third was made in the falx. The dura/falx/sinus complex was thus freed and lifted away from the cortex and draped over a bipolar platinum hook electrode. A bath of mineral oil, constrained by a dam of dental acrylic, was fashioned around the craniotomy to prevent the sinus and underlying cortex from drying out and to guard against current spread from the sinus to the cortex, which was additionally protected by a polyethylene cover inserted below the sinus. The sinus was stimulated via a stimulus isolation unit (Grass Instruments, SIU5A, Nattick, MA, USA) driven by a Grass S88 stimulator delivering single shocks (100 V ~1 mA, 250 μs duration) at a rate of 10/s (1 s on/1 s off). Local glucose utilization and blood flow were determined following 7–10 min of preconditioning stimulation. In some experiments the trigeminal ganglia were ablated electrically before SSS stimulation. The lesions were checked macroscopically after removal of the brain.

On-line monitoring

Blood pressure, FIO_2 and end-expiratory CO_2 signals were passed to a signal conditioning device and thence to both a chart recorder (Narco Bio-Systems, Narcotrace 80) for continuous display and to analog ports (LabMaster DMA, Scientific Solutions, Ohio) of an 80286/80287-based microcomputer (APC IV, NEC Biosystems, Tokyo, Japan) running under MSDOS 3.2 (Microsoft Co., USA). Signals were sampled continuously, using a program written in C (Kernighan and Ritchie, 1978), before and during stimulation and averaged over each respiratory pump cycle. Data were stored on disc and later analysed for any variation with stimulation. Experimental results were processed for plotting on a graphics printer (NEC P5320) or on a video screen driven by an EGA card.

Local blood flow

Local blood flow (LBF) was determined in specific areas of the brainstem and high cervical spinal cord using the freely diffusible tracer 4-[N-methyl-^{14}C]-iodoantipyrine (Amersham, England) and tissue autoradiography (Sakurada *et al.*, 1978), based on the theory of Kety (1951). The method is substantially similar to the dissection method that we have previously used (Goadsby and Duckworth, 1987). Following the period of preconditioning stimulation the tracer was infused, while stimulation continued at a constant rate for approximately 35 s with intermittent arterial blood sampling (100 μl) every 3–5 s. At the end of this period the animals were rapidly killed with a bolus of saturated KCl and the brain quickly removed and frozen with liquid freon. The blood samples were prepared for liquid scintillation counting by transfering 50 μl into glass scintillation vials, adding 1 ml of solubilizer (Protosol, New England Nuclear/ethanol solution 1.2) and incubating them in a shaking water bath for 1 h at 60° C. Radioactivity was determined, after the addition of a fluor (Biofluor, 15 ml; New England Nuclear), in a liquid scintillation spectrophotometer (Packard 300CD) with internal correction to disintegrations per min (dpm).

Serial frozen sections (20 μm) were taken from the caudal portion of the cervical segment of the cord to the midbrain using a sledge microtome in a freezing cabinet and placed on glass slides. These were exposed to Kodak SB-5 film in x-ray cassettes with ^{14}C labelled polymer standards (Amersham, England) for 2 wks and the film developed according to manufacturer's instructions. The tissue sections were processed for histology and stained with thionin. The autoradiograph standards were used to solve the Kety integral, and the autoradiographs of the brainstem and cord were then digitized with a dedicated image analysis minicomputer system (DEC PDP 11/73). Regions of interest were identified and outlined with the aid of a mouse and screen cursor so that average LBF could be determined in various central structures. At least 5 measurements were taken of each area in each animal and covered both the mediolateral and rostrocaudal extents of the regions.

Local glucose utilization

Local glucose utilization was determined using the technique of Sokoloff (Savaki *et al*, 1980) as applied in this laboratory (Goadsby, 1989). A 30–35 s pulse of 2-deoxy-D-[^{14}C]glucose (2DG) (Amersham, England) was administered intravenously accompanied initially by arterial blood sampling every 3–5 s (100 μl). This sampling continued at time intervals over the following 45 min, as did the stimulation of the SSS. At the conclusion of the infusion period the animal was killed and the brain rapidly removed and frozen with liquid freon and stored frozen until sectioning. The arterial blood was prepared for scintillation counting exactly as was the blood in the flow experiments except that 10 μl of plasma was also used to determine the free glucose concentration of the blood (Beckman Glucose-2). The brains were subsequently sectioned and autoradiographs produced as for the flow experiments. The standards and arterial concentrations of glucose and 2DG were used to solve the modified operational equation for varying glucose concentrations (Savaki *et al.*, 1980) using the lump constant for the cat (0.411) derived by Sokoloff (1979). Regions of interest were examined using computerized densitometry as in the flow experiments. The sections were also later stained with thionin for histological identification.

Experimental groups

Local blood flow and glucose utilization were studied in control animals in which the SSS was exposed and isolated but not stimulated, and in two experimental groups. In one group the SSS was stimulated electrically and in the other, both trigeminal ganglia were ablated by electrolytic lesions to block afferent vollies from the vessels and then the SSS was stimulated.

Statistics

Group data are expressed as mean ± SEM. A one-way analysis of variance (Walpole and Myers, 1978) was used to detect a difference in the entire cohort while a group variation from control was analysed using Dunnett's d test (Dunnett, 1955, 1964). Significance was assessed at the $P < 0.05$ level.

RESULTS

Control observations

A total of 22 cats of either sex were studied. The cardiovascular status and arterial blood gases of the animals analysed were within normal limits (Table 1) except for uniformly high arterial oxygen values that did not differ between experimental groups. The resting blood flows and glucose utilization data are included in Tables 2 and 3 and are well within normal limits for the brainstem and spinal cord (Nyström *et al.*, 1984; Edvinsson *et al.*, 1986; Goadsby and Duckworth, 1987). Areas of interest were selected based on the known anatomical and physiological connections of the sagittal sinus with the trigeminal system. Stimulation of the SSS itself did not alter cardiorespiratory factors significantly (fig. 1).

SSS stimulation

Stimulation of the SSS increased blood flow and glucose utilization at the cervicomedullary junction and in the spinal cord at the C2 level. The areas studied at the cervicomedullary junction are indicated in fig. 2 and those in the spinal cord at the C2 level in fig. 3, and the results given in Tables 2 and 3, respectively. In the region of the cervicomedullary junction, blood flow and glucose utilization were increased both in the trigeminal nucleus caudalis and in the ventral horn grey matter. Nucleus caudalis glucose utilization rose from a control level of 25 ± 5 μmol/100 g/min to

TABLE 1 PHYSIOLOGICAL RESULTS*

Group	*n*	*Weight (g)*	*Blood pressure*	*pH*	p_aCO_2	p_aO_2*
CBF						
Control	4	2782 ± 305	120 ± 5	7.36 ± 0.01	35 ± 1	314 ± 29
Stimulation	4	2810 ± 191	125 ± 6	7.37 ± 0.01	37 ± 1	300 ± 52
Trigeminal ganglia ablated[b]	3	2510 ± 220	118 ± 7	7.36 ± 0.02	36 ± 2	298 ± 38
CGU						
Control	4	2200 ± 99	109 ± 9	7.36 ± 0.01	38 ± 2	235 ± 31
Stimulation	4	2825 ± 261	124 ± 6	7.37 ± 0.01	36 ± 1	310 ± 49
Trigeminal ganglia ablated[b]	3	2100 ± 302	115 ± 4	7.35 ± 0.02	37 ± 1	281 ± 53

* Cardiovascular and blood gas parameters for groups of animals in all the experiments. All are within the physiological range except pO_2 (*) which is no different for either the control or stimulated groups. [b] Superior sagittal sinus stimulation with the trigeminal ganglia ablated bilaterally.

TABLE 2 SAGITTAL SINUS STIMULATION EFFECTS AT THE CERVICOMEDULLARY JUNCTION*

	Control (4)		*Stimulation (4)*		*Trigeminal ganglia (3)*	
Area	*BSBF*[a]	*BSGU*[b]	*BSBF*[a]	*BSGU*[b]	*BSBF*[a]	*BSGU*[b]
1	30±4	8±2	29±8	9±2	31±4	9±2
2	30±3	9±2	29±4	10±1	30±4	10±2
3	52±5	25±5	101 ± 9^{c}	90 ± 8^{c}	55±6	26±6
4	58±4	22±4	99 ± 9^{c}	88 ± 10^{c}	57±6	24±5
5	30±3	9±2	30±4	11±3	31±3	10±2
6	30±4	10±2	29±4	10±3	32±3	9±2
7	59±6	25±5	98 ± 8^{d}	78 ± 8^{d}	78 ± 6^{d}	83 ± 5^{d}
8	60±5	24±4	97 ± 8^{d}	79 ± 7^{d}	97 ± 5^{d}	87 ± 6^{d}
9	30±4	9±2	30±3	8±2	32±4	10±2
10	31±3	10±3	33±4	9±2	32±3	9±2

* The number of animals in each group is indicated in the respective columns. Fig. 2 illustrates the sites from which these data were obtained. [a] BSBF = brainstem blood flow (ml/100 g/min); [b] BSGU = brainstem glucose utilization (μmol/100 g/min); [c] Increased compared with control in the nucleus caudalis trigeminalis ($P < 0.01$) [d] Increased compared with control in the ventral grey matter ($P < 0.01$)

TABLE 3 SAGITTAL SINUS STIMULATION EFFECTS IN THE C2 SPINAL CORD*

	Control (4)		*Stimulation (4)*		*Trigeminal ganglia ablated (3)*	
Area	*SCBF*[a]	*SCGU*[b]	*SCBF*[a]	*SCGU*[b]	*SCBF*[a]	*SCGU*[b]
1	31±4	8±2	33±6	10±2	33±4	10±2
2	29±5	9±2	32±4	11±2	30±5	10±2
3	58±6	24±4	97 ± 8^{d}	52 ± 3^{d}	62±6	27±5
4	60±5	24±4	91 ± 9^{d}	52 ± 5^{d}	59±4	25±4
5	29±3	10±2	149 ± 18^{c}	78 ± 11^{c}	31±3	9±2
6	28±4	8±2	140 ± 9^{c}	83 ± 9^{c}	30±3	10±2
7	55±5	23±4	77 ± 6^{d}	35 ± 3^{d}	79 ± 5^{d}	35 ± 4^{d}
8	61±6	25±5	79 ± 5^{d}	36 ± 4^{d}	79 ± 6^{d}	36 ± 5^{d}
9	30±4	10±2	31±4	11±3	32±4	11±2
10	59±7	11±3	63±7	9±2	57±6	10±2

* The number of animals in each group is indicated in the respective columns. Fig. 3 illustrates the sites from which these data were obtained. [a] SCBF = spinal cord blood flow (ml/100 g/min); [b] SCGU = spinal cord glucose utilization (μmol/100 g/min), [c] Increased compared with control in the dorsolateral area of the C2 spinal cord ($P < 0.01$) [d] Increased compared with control in the spinal cord grey matter ($P < 0.05$).

90±8 μmol/100 g/min (an increase of 260%), with blood flow increasing in proportion ($d = 6.8$, $P < 0.01$); this increase was seen equally bilaterally. Both blood flow and glucose utilization were also increased bilaterally in the ventral grey matter although to a lesser extent (212% and 229%, respectively). No changes were seen in any white matter regions at this level or in the C1 level of the spinal cord.

At the C2 level of the cord, changes were observed in 3 distinct regions. First, both blood flow and glucose utilization were increased in the dorsal horn of the spinal cord, especially in the superficial laminae (areas 3 and 4; fig. 3). Blood flow increased by

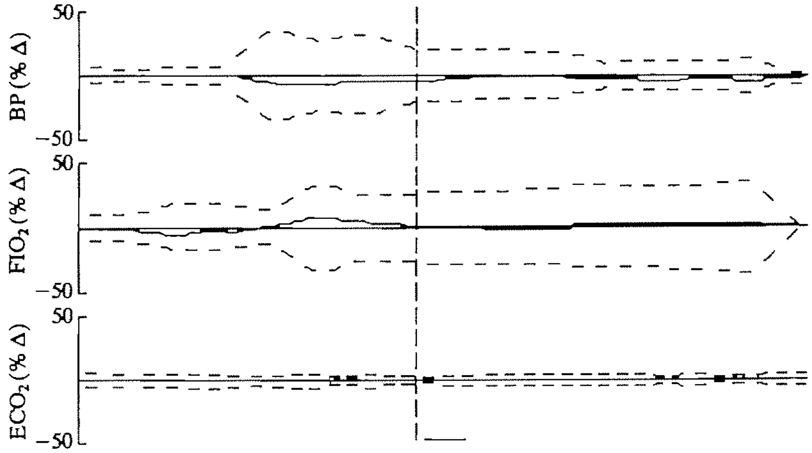

FIG 1 The mean percentage change in blood pressure (BP), end-expiratory CO_2 (ECO_2) and FIO_2 (O_2) averaged over the SSS stimulated animals and plotted with respect to time (abcissae). The dashed lines represent the 95% confidence interval envelopes for the physiological parameters monitored, the short continuous horizontal line is a time marker (75 s) and the vertical dashed line denotes the onset of SSS stimulation. There is no significant difference associated with stimulation for any of the physiological variables displayed.

67% and 52% and glucose utilization by 117% bilaterally. Similar, although quantitatively smaller, changes were seen in the ventral horn grey matter where glucose utilization increased by 52% and 32%, respectively. Both glucose utilization and blood flow were also increased in a small area adjacent to the dorsal horn (dorsolateral area, DLA). Glucose utilization increased to 78 ± 11 and 83 ± 9 μmol/100 g/min on either side accompanied by large increases in local blood flow (fig. 4). The size of this increase varied with the number of cells that the DLA contained, as determined from the subsequently stained cord sections. This variation in the number of cells also resulted in asymmetry of the changes in both blood flow and metabolism. Caudal to these cells no increased activity was seen in either the DLA or dorsal horn.

SSS stimulation with trigeminal ganglia ablation

In 3 animals in which the SSS was stimulated after both trigeminal ganglia had been ablated, no change in glucose utilization was found in the nucleus caudalis (d = 1.8, n.s.), the dorsal horn (d = 1.87, n.s.) or the DLA (d = 1.95, n.s.). The changes in the ventral horn grey matter were also unchanged (d = 1.92, n.s.). Some patchy changes in the intermediate grey matter were not significant when averaged over the cohort (Tables 2, 3).

DISCUSSION

These experiments have demonstrated that activation of a pain-sensitive structure, the superior sagittal sinus (SSS), can increase glucose utilization and blood flow in specific regions of the brainstem and spinal cord via a neural pathway that relays in the trigeminal ganglion. In these activated areas both blood flow and glucose utilization increased

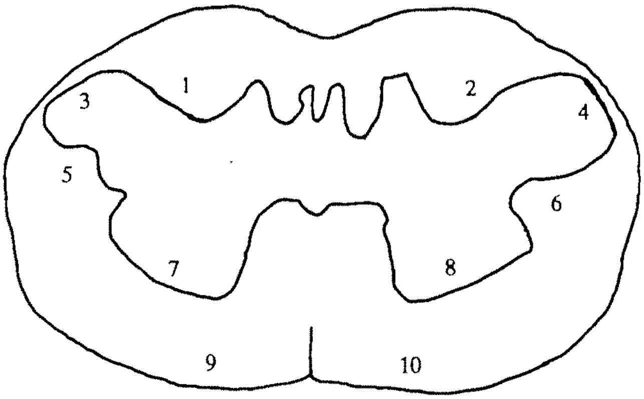

FIG 2. A line drawing from a histologically processed section of the caudal medulla at the level of the trigeminal nucleus caudalis indicating the positions from which the data in Table 2 were sampled.

Anatomical site	*Area*
Dorsal white matter	1, 2
Nucleus caudalis	3, 4
Intermediate white matter	5, 6
Ventral horn grey matter	7, 8
Ventral white matter	9, 10

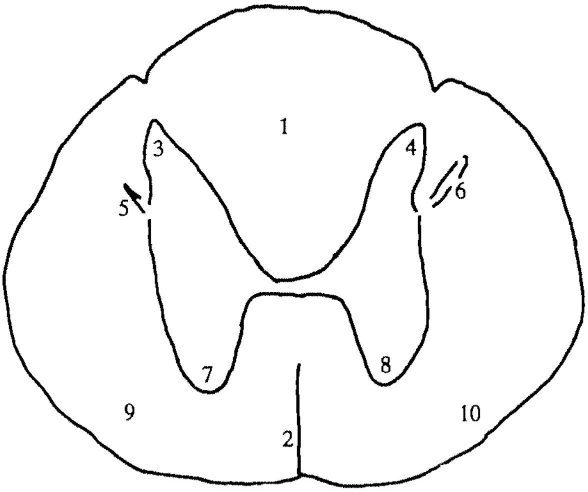

FIG. 3 A line drawing from a histologically processed section of the C2 level of the cervical spinal cord indicating the positions from which the data in Table 3 were sampled

Anatomical site	*Area*
Dorsal white matter (midline)	1
Dorsal grey matter (substantia gelatinosa)	3, 4
Dorsolateral area	5, 6
Ventral horn grey matter	7, 8
Ventral white matter	2, 9, 10

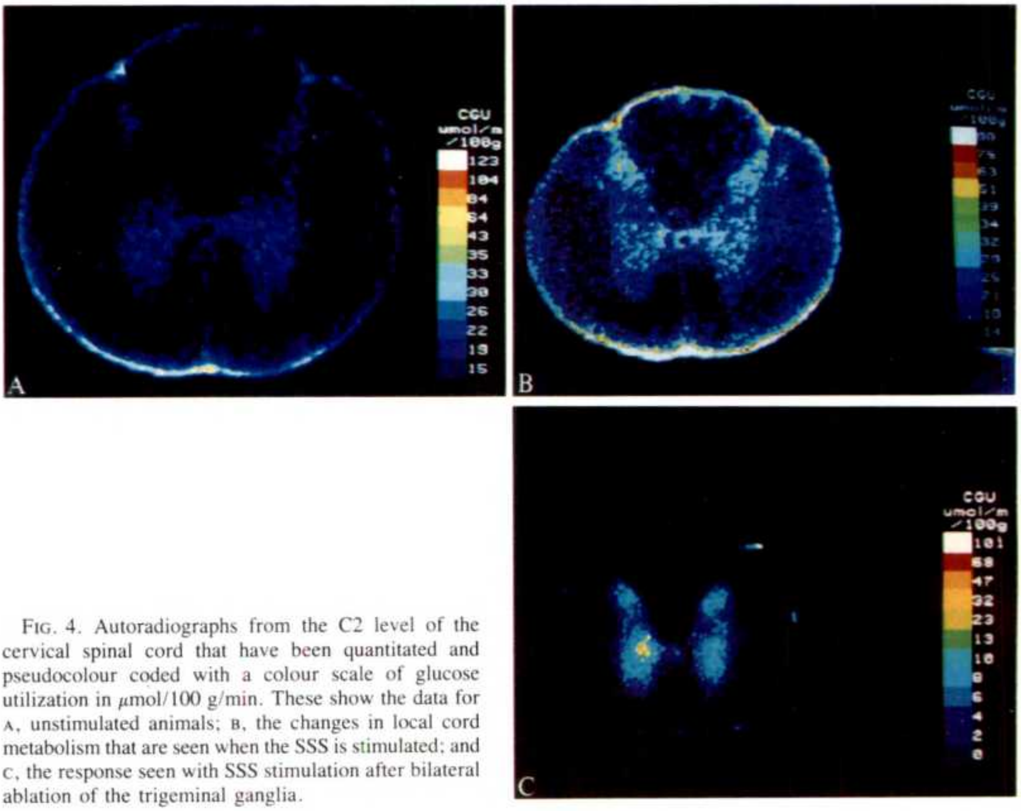

FIG. 4. Autoradiographs from the C2 level of the cervical spinal cord that have been quantitated and pseudocolour coded with a colour scale of glucose utilization in μmol/100 g/min. These show the data for A, unstimulated animals; B, the changes in local cord metabolism that are seen when the SSS is stimulated; and C, the response seen with SSS stimulation after bilateral ablation of the trigeminal ganglia.

together. Changes in blood flow thus followed changes in local glucose utilization, reflecting activation of the craniovascular nociceptive pathways.

Pain from the cranial vessels is transmitted predominantly through the ophthalmic division of the trigeminal nerve, the other divisions being involved to a lesser extent (McNaughton, 1966). The SSS is innervated by the trigeminal nerve, and is known to be pain sensitive in man (Grzybowski, 1931/1932; Penfield and McNaughton, 1940; Feindel *et al.*, 1960). The contribution of the trigeminal system to the innervation of the vascular structures of the posterior fossa, however, is small, with the main innervation being that from the upper cervical segments both in man (Grzybowski, 1931/1932) and cat (Kimmel, 1961; Keller *et al.*, 1985). Ultrastructural studies in the rat (Andres *et al.*, 1987) and human clinical data (Ray and Wolff, 1940) suggest that the afferent innervation of the dura and its vessels is primarily nociceptive and therefore should be relayed in the spinal trigeminal nucleus (Darian-Smith, 1973). Previous studies from this (Lambert *et al.*, 1979) and other laboratories (Davis and Dostrovsky, 1986; Strassman *et al.*, 1986) have demonstrated that electrical stimulation of the dural vessels and sagittal sinus activates neurons in the trigeminal nucleus caudalis. The experiments reported here extend these findings by demonstrating metabolic activation of the nucleus caudalis by stimulation of a central pain-sensitive structure, the SSS, thus confirming the importance of the trigeminal system in nociceptive transmission from the cranial vessels. The blockade of this response following ablation of the trigeminal ganglia (bilaterally, because the SSS is a midline structure) demonstrates that this response was specifically mediated by the trigeminal system and not primarily caused by spread of current to underlying cortex with subsequent activation of a descending corticospinal pathway. The persistence of increased ventral horn and in some animals intermediate grey matter activity indicates activation of nontrigeminal pathways that may include some current leakage.

Previous experiments from this laboratory (Lambert *et al.*, 1986, 1988) have identified an area of the upper cervical spinal cord in which large field potentials could be obtained by stimulation of the SSS. For this reason observations of blood flow and metabolism were extended to the C2 level of the cord. In the dorsolateral area adjacent to the dorsal horn but in the white matter, a group of cells was consistently activated by stimulation of the SSS. This area also took its input from the trigeminal system since ablation of the trigeminal ganglion blocked the increase in activity elicited with SSS stimulation.

What then is the nature of this unusually located responsive region? The cells described are certainly located somewhat caudal to the nucleus caudalis as it is usually defined (Darian-Smith, 1973). Clinical experience has shown that trigeminal tractotomy at, or near, the obex impairs pain sensation on the face while leaving tactile sensation relatively spared (Gerard, 1923; Sjöqvist, 1939). This is supported by histological data demonstrating that most unmyelinated nerves terminate in the nucleus caudalis whereas large myelinated fibres end in the more rostral subnuclei (oralis and interpolaris) of the trigeminal complex (Wall and Taub, 1962; Darian-Smith *et al.*, 1965). Fibres responsible for perioral and intraoral facial nociception provide a well documented exception to this rule as they terminate rostrally (Hayashi *et al.*, 1984). In addition, Arbab *et al.* (1988) have used tracer techniques in the rat to demonstrate a group of cells in the dorsal horn, discontinuous with the trigeminal complex, that receives an input from the cerebral vessels. Edvinsson *et al.* (1989) have demonstrated labelling of the C2 dorsal ganglia of the rat and more recently in the monkey (L. Edvinsson, personal communication) highlighting the importance of this region. The active area identified in these experiments in the cat may well correspond to the anatomically localized group of cells in the region that has been labelled as the lateral cervical nucleus (Rexed, 1952). Although this nuclear group has previously been associated with the relay of tactile information from the limbs (Willis, 1985), more recent data have demonstrated that it may have a role in nociception (Kajander and Giesler, 1987).

In summary, the distribution of neurons activated by craniovascular nociception has been mapped in the caudal medulla and upper cervical spinal cord of the cat. In accordance with previous anatomical data, metabolism and blood flow in the nucleus caudalis increased when a pain-sensitive intracranial vascular structure, the SSS, was stimulated. In addition, a group of cells in the dorsolateral area of the second cervical segment of the spinal cord, including the dorsal horn, became highly active during stimulation of the SSS. The relationship of these cells to those previously studied in the lateral cervical nucleus remains to be determined. Both caudalis and DLA responses were blocked by ablation of the trigeminal ganglia, indicating that their input was via the trigeminal nerve. An understanding of the anatomical connections, physiology and pharmacology of these nuclear groups is fundamental to an understanding of the pathophysiology of migraine and to the rational development of therapy for headache.

ACKNOWLEDGEMENTS

The authors thank Professor James Lance for his continued support and critical review of the manuscript and Dr Geoffery Lambert, Hark Hellier and Paul Charalambous for their assistance. The authors also thank the Department of Medical Illustration, University of New South Wales, for photographing all figures. This programme has been supported by the National Health and Medical Research Council of Australia and by grants from Warren and Cheryl Anderson, the J. A. Perini Family Trust, the Basser Trust and the Australian Brain Foundation This work was presented in part at the 14th International Symposium on Cerebral Blood Flow and Metabolism, Bologna, Italy, 1989 (Goadsby *et al.*, 1989).

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(*Received December 28, 1989. Revised June 5, 1990. Accepted June 26, 1990*)

Brain (1991), **114**, 1013–1023

HISTOPATHOLOGY OF MULTIPLE SCLEROSIS LESIONS DETECTED BY MAGNETIC RESONANCE IMAGING IN UNFIXED POSTMORTEM CENTRAL NERVOUS SYSTEM TISSUE

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SUMMARY

Postmortem unfixed whole brains from 17 multiple sclerosis and 6 control cases were examined by magnetic resonance imaging (MRI) using a T_2-weighted spin echo sequence and histology to determine the relationship between areas of abnormal MRI signal and underlying pathological change. In group 1, small MRI lesions and correspondingly small plaques, most of which were chronic, were detected histologically in 5 brains. In 4 brains there were more extensive areas of both abnormal signal and histological plaques which were more often active (group 2). However, in a further 5 brains extensive MRI abnormalities were observed when only small periventricular plaques were present histologically (group 3). Lesions in the hindbrain and cerebral grey matter were infrequently observed by MRI. The extensive MRI abnormalities seen in areas in which only small histological plaques were found may be the result of vascular permeability changes in the normal-appearing white matter surrounding plaques.

INTRODUCTION

Magnetic resonance imaging (MRI) of the brain and spinal cord of patients with multiple sclerosis (MS) is being used increasingly in the clinical evaluation of demyelinating disease. The MRI signal in the normal central nervous system (CNS) is largely derived from protons in water, and in MS abnormal signals are related to an increase in tissue water and to its protein content and cellular location (Barnes *et al.*, 1987*a, b*; Ormerod *et al.*, 1987). In vivo, a range of abnormal MRI signals is seen in lesions (Ormerod *et al.*, 1987; Paty and Li, 1988), and areas of abnormal signal which change in size have been observed in serial studies (Koopmans *et al.*, 1989; Kermode *et al.*, 1990). Despite this, the exact relationship between abnormal signal areas and the histopathological characteristics of individual lesions is unclear.

In studies on a small number of postmortem MS brains fixed in formaldehyde, areas of abnormal MRI signal have been shown to correspond broadly to plaques identified microscopically (Stewart *et al.*, 1986; Nagara *et al.*, 1987; Ormerod *et al.*, 1987; McConnell *et al.*, 1989). However, fixation alters tissue water distribution, chemical

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composition (Stewart *et al.*, 1986), brain image contrast and reduces the MRI parameters T_1 and T_2 (Nagara *et al.*, 1987). Formaldehyde fixation also appears to prevent MRI detection in early active plaques in the brains of primates with experimental allergic encephalomyelitis (Paty and Li, 1988).

To explore further the relationship between MRI and pathological changes we have scanned postmortem unfixed whole brain and spinal cord from patients with MS. The distribution of lesions detected by MRI and those observed macroscopically at dissection were compared, and tissue sections were examined by light microscopy after histological and immunocytochemical staining to identify active and chronic plaques. The results have been reported in abstract form (Newcombe *et al.*, 1989*a*, *b*).

METHODS

MRI scanning

Postmortem unfixed CNS tissue from 17 cases diagnosed clinically as MS by the referring neurologist and subsequently confirmed by histology was obtained from the Multiple Sclerosis Tissue Bank at the Institute of Neurology. The average age of the MS patients was 61 (range 40–76) yrs, the duration of clinical disease was 23 (range 3–50) yrs and the time interval between death and imaging was 34 (range 10–63) h. Unfixed brains from 6 postmortem control cases were also scanned (average age 52, range 23–87) yrs, and the interval between death and imaging was 41 (range 30–62) h. Of these 6 cases, 3 were normal controls with no known neurological disease, 2 were diagnosed clinically as possible cases of MS but no MS pathology was seen at dissection or microscopically, and 1 had CNS sarcoidosis.

Brain and spinal cord were placed in plastic bags and packed in wet ice for transport to the scanner. The dorsal surface of each whole brain was placed on the bottom of a plastic container and the brain was covered with ice and scanned with a 0.5 Tesla Picker International imager. After a pilot scan in the transverse plane, 24 contiguous coronal images with a slice thickness of 5 mm were obtained using a T_2-weighted spin echo sequence (SE 4000/120—2 repetitions, 128 values of phase-encoding gradient and 25 cm field of view with pixel size 2×2 mm). Spinal cords from 10 of the MS cases were cut transversely into 4 lengths and scanned in the transverse (axial) plane on a rigid plastic sheet, using a specially designed surface coil. The spin echo sequence was adjusted accordingly (SE 2000/80–4 repetitions, 256 values of phase-encoding gradient with pixel size 1×1 mm).

All brain MRI scans were evaluated 'blind' by 2 independent assessors (C.P.H., E.P.G.H. du B.). The number of areas of abnormal signal were counted in the frontal, parietal, temporal and occipital regions, and in the cerebellum, pons and medulla of each brain. To facilitate comparison of lesions seen on coronal MRI with plaques observed at dissection, the division between the frontal and parietal regions was taken as a vertical plane from the sylvian fissure and the division between the parietal, temporal and occipital regions as a vertical plane at the trigone.

Areas of abnormal signal on MRI in each region were counted in two ways. The first was to record the number of separate areas of abnormal signal, whether heterogeneous in appearance or not. In some instances one area of abnormal signal was accompanied by another adjacent area, but the latter was only considered to be separate if no connection to a previously counted area was seen in other slices. The second way of counting lesions was to make a detailed assessment of the maximum possible number of high signal areas. These were either (1) focal lesions or highlights within a periventricular haze of relatively lower abnormal signal or (2) focal lesions outside the haze.

The effect of postmortem changes on areas of abnormal signal was examined in 1 MS case and 2 control cases, 1 of which was a normal control and the other a possible MS case in which no MS pathology was present. After the cerebral and cerebellar hemispheres were separated by cutting along the midline, the 2 hemispheres were scanned together. Samples from a cerebral and cerebellar hemisphere of each case were dissected and snap-frozen after scanning. The other hemispheres were kept at 4° C and scanned on the fourth and seventh days. The tissue was then dissected and samples were snap-frozen for cryostat sectioning.

Dissection and histological staining

After scanning, brain and spinal cord were dissected on ice and examined macroscopically to locate plaques. From 4 MS cases only 1 cerebral and cerebellar hemisphere and part of the spinal cord were available for macroscopic and microscopic examination as tissue was taken for routine histology. An average of 70 blocks (approximately 1 cm^3) of plaque and macroscopically normal tissue were dissected from the cerebrum, hindbrain and spinal cord of each case. The blocks were placed in OCT Compound (BDH, Poole, UK) on cork discs, snap-frozen in isopentane cooled on liquid nitrogen and stored at $-70°$ C. Cryostat sections (10 μm) were cut from the blocks and stained with haematoxylin and eosin to assess cellular infiltration and with oil red O to demonstrate degenerating myelin. Sections from all blocks were incubated with monoclonal antibodies directed against the myelin lipid galactocerebroside (Ranscht *et al.*, 1982) to demonstrate myelin loss in plaques, and against the astrocyte cytoskeletal glial fibrillary acidic protein (GFAP) (Newcombe *et al.*, 1986) to show astrocyte scar formation. After immunoperoxidase staining with an avidin-biotin system (Vector Laboratories, Peterborough, UK) the haematoxylin counterstained sections were dehydrated and mounted in DPX (BDH).

RESULTS

In unfixed postmortem MS brains the distribution and appearance of areas of abnormal MRI signal in the transverse and coronal planes and also the range of T_1 and T_2 values for apparently normal white matter (T_1 = 311 − 466, and T_2 = 62 − 118) and for lesions (T_1 = 383−745 and T_1 = 86−527) were similar to those seen in MS patients in vivo (C. P. Hawkins, unpublished results). Small periventricular areas of abnormal signal were observed in the brain from an 87-yr-old normal control case, but no other areas of abnormal MRI signal were seen in the 6 control brains (fig. 1). No macroscopically abnormal tissue was seen at dissection and no pathological abnormalities were found on microscopic examination of sections of the brain from the 87-yr-old normal control or any of the other control brains.

One cerebral and cerebellar hemisphere from 1 MS and 2 controls were scanned 3

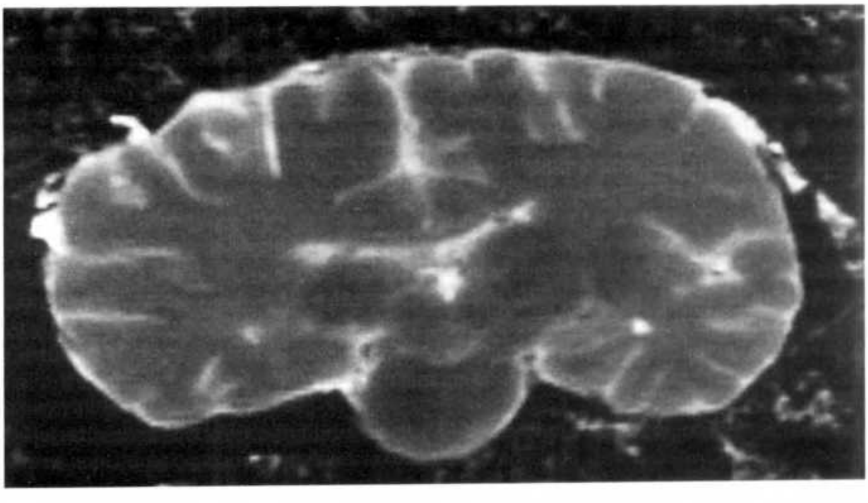


FIG. 1. MRI coronal slice from the parietal region of a postmortem unfixed control brain scanned with a T_2-weighted spin echo sequence (SE 4000/120), slice thickness 5 mm; the brain was packed in wet ice. No areas of MRI or histological abnormality were seen in this brain.

times in 7 days. In the MS brain extensive areas of abnormal MRI signal were observed but only small periventricular plaques were found at dissection, and in the normal control brain from the 87-yr-old subject mentioned previously small periventricular areas of abnormal signal were seen. In the 7 day period there was no change in the number,

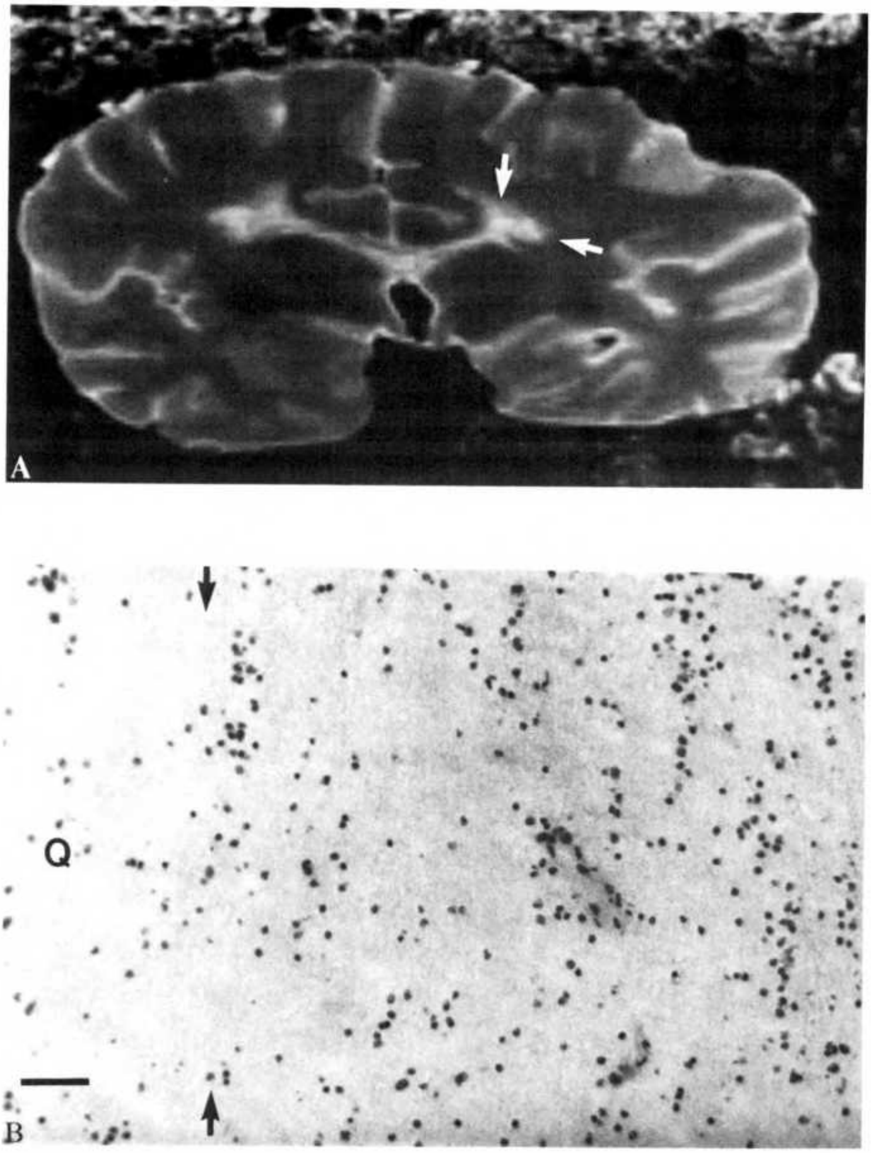


FIG. 2. A, MRI coronal slice from the parietal region of a postmortem unfixed MS brain showing regions of periventricular high signal, from *Case 2* in group 1. The arrows point to the periventricular abnormality seen in the right hemisphere; there are similar changes in the opposite hemisphere. B, cryostat section from the edge (indicated by the arrows) of a periventricular hypocellular chronic plaque corresponding to the area of periventricular high signal on MRI shown by arrows in A. Haematoxylin and eosin. Bar = 50 μm.

size or density of any of the areas of abnormal signal in the hemispheres from these cases.

Tissue sections of the plaques with perivascular inflammation, hypercellularity and oil red O positive degenerating myelin were considered to be actively demyelinating, while hypocellular galactocerebroside-negative demyelinated plaques with dense GFAP-stained astrocyte scars were classified as chronic inactive plaques (figs 2B, 3B, 4B). Grey matter plaques were characterized microscopically by the presence of astrogliosis.

In the 14 cases in which cerebral MRI lesions were observed, most of the areas of abnormal signal were bilateral in the cerebral hemispheres. Four categories of lesion distribution were seen in the periventricular white matter of the 17 MS cases.

1. In 5 MS brains, small separate MRI abnormalities with a depth of 1−4 mm from the ventricular surface were seen where plaques of 1−3 mm depth were observed at dissection and histologically (group 1 in the Table; fig. 2). There was a statistically

TABLE. MRI AND HISTOLOGICAL ANALYSIS OF PERIVENTRICULAR MS LESIONS

*Group 1: Limited MRI and histological abnormalities. Range of MRI lesion depth 1−4 mm, plaque depth 1−3 mm***

Case no.	1*	2	3*	4*	5	Mean ± SD
No. of separate MRI lesions	6	9	10	14	7	9.2 ± 3.1
Maximum no. of MRI lesions	14	16	24	14	22	18.0 ± 4.7
% as focal lesions in signal haze	0	19%	0	0	36%	
Histological plaques	12	5	14	24	19	14.8 ± 7.2
Percentage active	17%	0	0	25%	26%	
Tissue blocks examined	58	35	20	64	35	

*Group 2: Extensive MRI and histological abnormalities. Range of MRI lesion depth 2−24 mm; plaque depth 5−20 mm***

Case no.	6	7	8	9	Mean ± SD
No. of separate MRI lesions	11	8	15	12	11.5 ± 2.9
Maximum no. of MRI lesions	40	42	47	82	52.8 ± 19.7
% as focal lesions in signal haze	77%	50%	19%	52%	
Histological plaques	23	26	31	13	23.3 ± 7.6
Percentage active	9%	69%	71%	31%	
Tissue blocks examined	60	30	31	23	

*Group 3: Extensive MRI but limited histological abnormalities. Range of MRI lesion depth 2−24 mm; plaque depth 1−2 mm***

Case no.	10*	11	12	13	14	Mean ± SD
No of separate MRI lesions	10	10	8	11	14	10.6 ± 2.2
Maximum no of MRI lesions	64	30	47	33	42	43.2 ± 13.5
% as focal lesions in signal haze	69%	0	77%	67%	74%	
Histological plaques	12	9	10	13	9	10.6 ± 1.8
Percentage active	33%	22%	50%	8%	11%	
Tissue blocks examined	84	37	28	23	41	

* Only 1 hemisphere was dissected and sectioned; the other was taken for routine pathology; data expressed per whole brain. ** MRI lesion and plaque depths were measured from the ventricular surface.

significant difference between mean numbers of separate (9.2 ± 3.1) and maximum (18.0 ± 4.7) MRI lesions ($P < 0.01$; nonpaired Student's t test) in this group, but there was some variability in numbers of histological plaques in comparison with MRI lesion counts. Focal high signal areas in a haze were observed in only 2 brains, and the majority of group 1 plaques were chronic.

2. In the periventricular white matter of the 4 MS brains in group 2 the depths of MRI abnormal signal areas and histological plaques were greater than in group 1, ranging from 2 to 24 mm for abnormal signal areas and 5 to 20 mm for plaques (group 2 in the Table; fig. 3). The mean number of separate MRI lesions (11.5 ± 2.9) was similar to that in groups 1 and 3. However, in group 2 there were significantly more histological plaques (23.3 ± 7.6) than separate MRI lesions ($P < 0.05$), and more MRI maximum lesions (52.8 ± 19.7) than either the histological plaques ($P < 0.05$) or separate MRI lesions ($P < 0.01$). The number of focal lesions in an abnormal signal haze ranged from 19% to 77%, and perivascular inflammation and active demyelination was most pronounced in the plaques in this group.

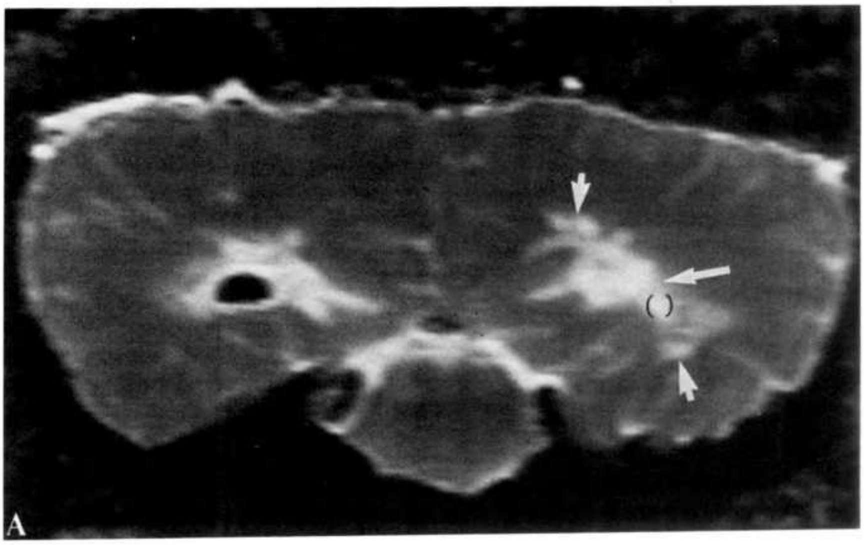
3. In a further 5 brains extensive abnormal signal areas as large as those seen in group 2 were observed, and in some slices from these brains the MRI abnormalities extended from the periventricular surface to the grey-white matter borders of the cerebrum (group 3 in the Table; fig. 4). The mean number of separate MRI lesions and histological plaques was the same, 10.6, although only small periventricular plaques less than 2 mm in depth were present in macroscopically and microscopically normal white matter within the extensive abnormal signal areas. In the maximum assessment the mean number of MRI lesions (43.2 ± 13.5) was significantly greater ($P < 0.001$) than that of either the separate lesions or the histological plaques. Over two-thirds of the maximum MRI lesions were focal in 4 brains in this group. In groups 1 and 2 the focal lesions were within plaques, but these lesions were present in the white matter of group 3 brains.

4. In 3 MS cases, MRI abnormalities and histological plaques were found in the spinal cord although none were detected in the brain.

Using the T_2-weighted spin echo sequence no lesions were seen in the corpus callosum by MRI on coronal and transverse slices, although a sagittal image would have been optimal for detecting callosal abnormalities. A total of 26 callosal plaques was detected pathologically in 6 cases. Fifteen central grey matter plaques were observed at dissection and histologically in 8 brains but only 2 of these were detected by MRI. None of the 39 cortical plaques identified histologically in 8 brains were detected by MRI. Out of a total of 75 plaques present in the pons, medulla and cerebellar white matter of 13 MS cases only 8 were detected by MRI. Most plaques in grey matter and in the cerebellum, pons and medulla were between 1 and 5 mm in size. Abnormalities were detected with transverse MRI using a surface coil in all of the 10 MS spinal cords. Lesions as small as 5 mm in diameter could be detected and there was a good correlation between MRI abnormalities and histology.

DISCUSSION

The distribution and appearance of areas of abnormal MRI signal in postmortem MS brains were found to be comparable with those seen in MS patients in vivo. Although biochemical and physical changes which affect MRI parameters might be expected to



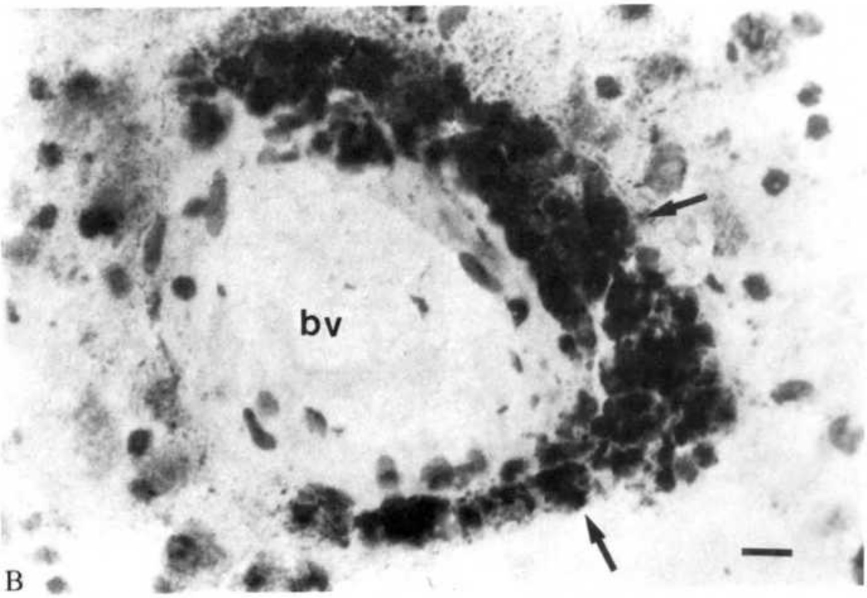


FIG. 3. A, MRI coronal slice from the occipital region of MS *Case 8* in group 2 showing a periventricular haze of abnormal signal containing several focal highlights. The large arrow points to the periventricular haze; the brackets indicate a focal lesion within the haze and the small arrows demonstrate focal lesions outside the haze in the right hemisphere. Similar changes are seen in the opposite hemisphere. B, cryostat section from an actively demyelinating periventricular plaque from the region of abnormal signal on MRI shown by the large arrow in A; stained with oil red O and haematoxylin to show an inflammatory cuff with oil red O staining of degenerating myelin in macrophages (indicated by the arrows) around a blood vessel (bv). Bar = 10 μm.

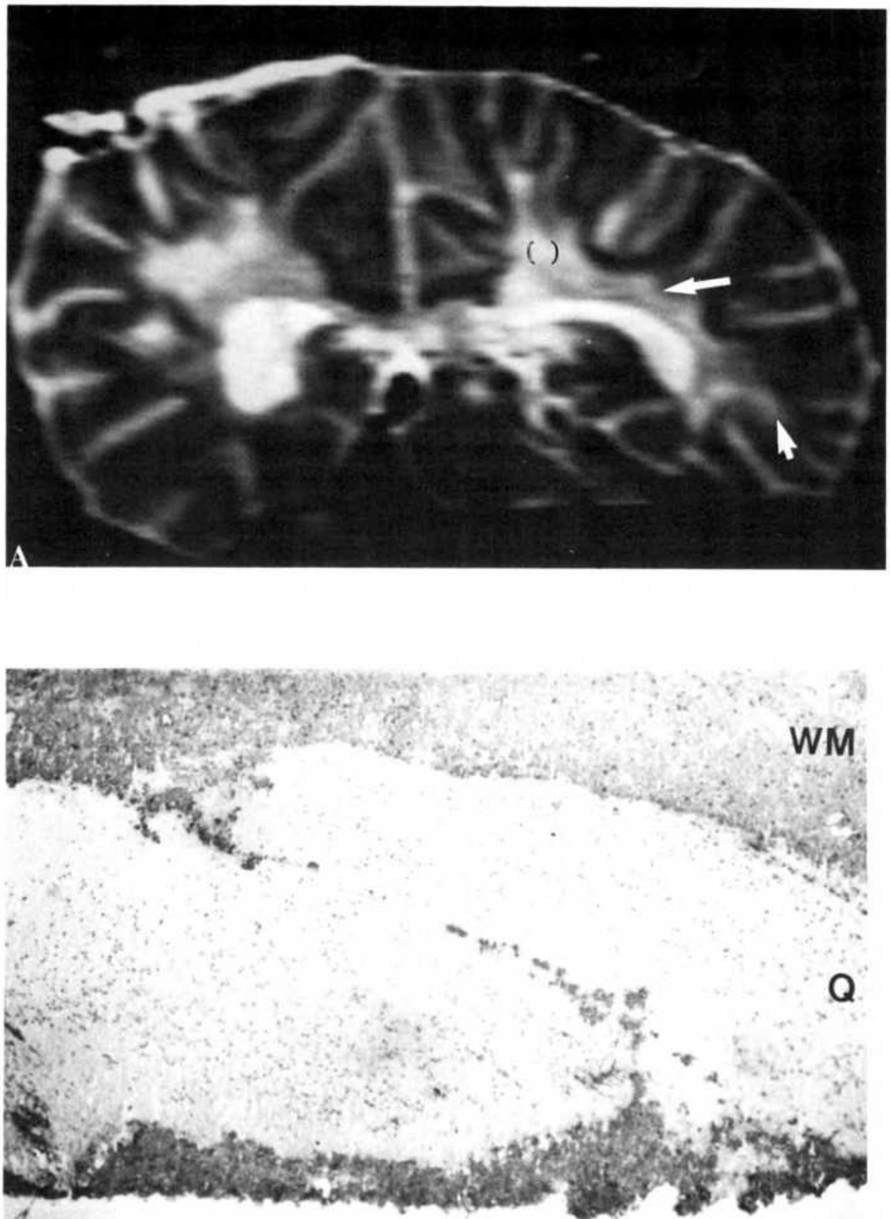


FIG. 4. A, MRI coronal slice from the occipital region of MS *Case 12* in group 3, with extensive areas of abnormal MRI signal. The large arrow points to the periventricular haze, the brackets indicate a focal lesion within the haze and the small arrow a focal lesion outside the haze in the right hemisphere. Similar changes are seen in the opposite hemisphere. B, cryostat section of a shallow periventricular chronic plaque surrounded by apparently normal white matter which extended up to the grey-white matter borders of the cerebral cortex; this section was from the periventricular region of abnormal signal on MRI indicated by the large arrow in A. Immunoperoxidase staining with antigalactocerebroside monoclonal antibody to show apparently normal white matter (WM) surrounding the demyelinated plaque (Q) related to the ventricle surface (V). Bar = 100 μm.

occur in tissue shortly after death, no relationship was found between the death-scanning interval and the extent of abnormal signal, and no qualitative differences were detected in the MS and control brains scanned serially over 7 days.

The number of separate MRI lesions was either less than or equal to the number of plaques identified by histology in the cerebrum in most of the MS cases. However, in the assessment of the maximum possible number of MRI lesions, the total often exceeded the number of histological plaques. Lesions in the cerebral cortex, thalamus and hindbrain were infrequently visualized with the standard T_2-weighted images of the head, although the dimensions of the lesions at dissection were generally similar to those detected in the periventricular white matter by MRI. While most may not have been detected because of the difficulty of distinguishing surface plaques from partial volume artefacts, some plaques which were not visualized on the scans were located deep within the thalamus, brainstem or cerebellum.

The importance of scanning the spinal cord in MS patients in vivo is emphasized by the detection of pathology in the cord by transverse MRI and histology in 3 MS cases in which no lesions were seen in the brain with either technique. The usual method of sagittal MRI of the spinal cord in vivo may result in an underestimation of the number of lesions because the characteristic longitudinally-running narrow lateral column plaques would be most readily detected by scanning in the transverse plane.

There is evidence from in vivo measurements of T_2-magnetization decay curves in experimental animals (Barnes *et al.*, 1987*a*) and patients with MS (Barnes *et al.*, 1987*b*) and from electron microscopic morphology in both (D. Barnes *et al.*, unpublished results) which strongly suggest that chronic MS lesions are heterogeneous and are made up of varying proportions of demyelinated axons, astrocytic processes and an expanded extracellular fluid space of low protein content. This multiplicity of pathological features probably accounts for the variability in lesion detection when comparing MRI and histology in the chronic cases in group 1. However, when the pathology was more extensive and inflammatory in nature, as exemplified by the cases in group 2, the correlations between MRI and histological abnormalities were less variable, demonstrating the value of assessing high signal areas within a periventricular haze at MRI. There is thus a group of postmortem brains which show small MRI lesions and correspondingly small histological plaques which are usually chronic (group 1). In contrast, there are brains which reveal more extensive areas of abnormal signal, frequently heterogeneous in appearance, where there are either equivalent regions of histological abnormality often containing active plaques (group 2) or where the plaques are small and discrete (group 3). It seems likely that the abnormal signal seen on MRI in the latter groups is also related to an enlarged extracellular space, with or without gliosis.

Our observations of extensive areas of abnormal signal in postmortem tissue where only small plaques were seen macroscopically and microscopically could be related to changes in vascular permeability in the white matter. Periventricular areas of abnormal MRI signal which generally have smooth outlines are seen in cerebral vascular disease and often in elderly individuals (Ormerod *et al.*, 1987; Gerard *et al.*, 1989), as reported for 1 case in this study. However, there are several lines of evidence to suggest that the extensive areas of high signal we have observed at MRI in the MS cases of group 3 in the absence of comparable pathological change are a part of the disease process in MS. MRI lesions can increase and decrease in size and intensity over several weeks on serial scans (Miller *et al.*, 1988; Koopmans *et al.*, 1989; Kermode *et al.*, 1990). Large MRI lesions are seen in patients with benign MS (Thompson *et al.*, 1989). Gadolinium-DTPA (Gd-DTPA) enhancement of MRI abnormalities in MS patients and animals with demyelinating disease when the process is active indicates blood-brain barrier breakdown in inflammatory lesions (Miller *et al.*, 1988; Hawkins *et al.*, 1990; Kermode *et al.*, 1990) for which there is support from postmortem evidence of permeability changes in the vessels in and around plaques (Broman, 1964; Adams, 1988).

It is thus probable that the extensive areas of abnormal signal on MRI seen in the MS cases in group 3 result from vascular permeability changes due to the disease process, both in active and chronic plaques, and in the normal-appearing white matter. This would lead to an increase in the extracellular water content of plaques and the adjacent tissue surrounding the plaques. These postmortem observations provide support for the view that the quickly-resolving diffuse areas of high signal seen in vivo around lesions known from serial scanning and the presence of Gd-DTPA enhancement to be new are due to oedema.

There is increasing evidence to suggest that MS is a more diffuse process than has been previously thought. In vivo MRI T_1 and T_2 relaxation times are significantly higher in normal-appearing white matter in MS patients than controls (Miller *et al.*, 1989), and widespread gliosis with or without scanty multifocal demyelination is often seen in macroscopically normal white matter from postmortem cases of MS (Newcombe *et al.*, 1980, 1986; Allen *et al.*, 1981). We are currently studying T_1 and T_2 relaxation times in plaques and macroscopically normal white matter in MS postmortem tissue in conjunction with a detailed histopathological assessment of the degree of inflammation, demyelination, gliosis and blood vessel wall damage in order to elucidate further the origin of abnormal MRI signal in MS and determine the usefulness of quantitative data in differentiating active inflammatory lesions from chronic inactive plaques.

ACKNOWLEDGEMENT

This study was supported by the Multiple Sclerosis Society of Great Britain and Northern Ireland.

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(Received December 28, 1989. Revised June 12, 1990. Accepted June 26, 1990)

Brain (1991), **114**, 1025–1043

PROCESSING OF SPATIAL RELATIONS WITHIN AND BETWEEN THE DISCONNECTED CEREBRAL HEMISPHERES

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SUMMARY

This study examined three issues related to the processing of spatial relations by commissurotomized subjects. One issue was concerned with the respective competence of the disconnected cerebral hemispheres at performing judgements of relative position and distance between 2 objects appearing at varying locations in the visual space. Contrary to recent suggestions, the results showed that the two hemispheres were equally competent at representing categorical and coördinate spatial relations and at operating on these representations. The second issue was concerned with the capacity of the two disconnected hemispheres to operate conjointly. The subjects were simultaneously presented with the same information in the two visual fields and were required to produce a single response based on this information. Compared with unilateral presentations, bilateral presentations resulted in enhanced response accuracy and, depending on the type of decision, in patterns of response latency and accuracy different from the patterns of either unilateral condition. These results suggest that the two disconnected hemispheres can operate simultaneously and are able to join the outcomes of their respective operations before the production of a single response. The third issue was concerned with interhemispheric communication of visuospatial information. Unlike pattern information that is typically confined to the hemisphere that receives it in the commissurotomized brain, visuospatial information could be subjected to interhemispheric comparison as a function of its categorical and metric properties, although the patients had only implicit knowledge of part of the transferred information. Implications of these findings for the contribution of the hemispheres to visuospatial processing and for the understanding of the functioning of the commissurotomized brain are discussed.

INTRODUCTION

The unique neurological condition of the human commissurotomized brain has served neuropsychological research in two broad categories of questions. One has been concerned with the functional specialization of the cerebral hemispheres, the study of which can be carried out within the same brain with little interhemispheric interference as a result of the section of the main means of communication between the two hemispheres. In spite of abnormalities of the patients' brains, and of individual differences among patients, the general findings essentially confirmed and extended the knowledge acquired from classical neurology and opened the way to further elaborations and specifications of the role of the cerebral hemispheres in controlling behavioural and cognitive functions (Sperry, 1970, 1984; Levy and Trevarthen, 1976; Gazzaniga and LeDoux, 1978; Bogen, 1986). The second broad category of questions has been directed at examining the effects of cutting the neocortical commissures on the functioning of the whole brain, considering the negative signs that form the disconnection syndrome (e.g., Bogen, 1985) and the

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positive signs that result from interhemispheric communication through subcortical structures (Trevarthen and Sperry, 1973; Sergent, 1983, 1990; Holtzman, 1984). These two main issues will be addressed in the present study which is specifically concerned with examining hemisphere processing of spatial relations and with investigating interhemispheric communication of visuospatial information and the conjoint functioning of the two disconnected hemispheres.

Hemisphere contribution to the processing of spatial relations

There is a large body of evidence pointing to a crucial role of the right hemisphere (RH) in the processing of spatial information (De Renzi, 1982; Benton, 1985). This applies to the localization of points in space, judgement of direction and depth, topographical orientation, 'manipulospatial' skills, and transformation of mental images. Another aspect of spatial processing has recently received special attention in the context of a computational theory of visual perception. Kosslyn (1987) has suggested that two broad kinds of spatial relation representations are used in human vision. One type of representation captures general properties of spatial structures irrespective of specific metric properties, and it describes *categorical* relations among objects or parts of objects. The other type of spatial relation is concerned with precise metric information about objects in space, and it describes *coordinate* relations in which locations of objects are specified in terms of metric units. Kosslyn further suggested that, because categorical and coordinate representations are qualitatively different, separate processing subsystems must produce each type of representations. In addition, referring to the role of the left hemisphere (LH) in categorical perception of speech and to the critical contribution of the RH to navigation and orientation, Kosslyn proposed that the two spatial relation representations have different cerebral bases, and, therefore, are genuinely distinct.

Testing this hypothesis on normal subjects in divided visual field experiments, Hellige and Michimata (1989) and Kosslyn *et al.* (1989) found confirmation of the predictions and observed a RH superiority in the judgement of distance between 2 objects and a LH advantage for processing categorical spatial relations. Because the designs of these studies could lead to alternative interpretations of the findings, Sergent (1991) conducted a series of experiments that further examined this issue and found no evidence of hemisphere specialization for processing the two types of spatial relation representations. In fact, these two kinds of spatial relation, at such an elementary level, are intrinsic to visual processing and are such fundamental properties of vision that it is hardly conceivable that either hemisphere would be bereft of one type of representation. For example, tasks such as calculation and recognition of noncanonical views of objects, predominantly subserved by the LH and the RH, respectively, cannot be efficiently performed without taking account of the position of component parts relative to one another. This is not to say that the two hemispheres must be equally efficient at making the two different types of spatial relation judgements, but it is an empirical question to determine whether or not 'categorical' and 'coordinate' spatial relation information is contained in separate and distinct representations, and whether or not each hemisphere is equipped for representing only one type of spatial relation.

Examination of the capacity of the disconnected hemispheres to perform judgements of distance and relative position may thus provide information about the respective competence of the cerebral hemispheres at producing, and operating on, spatial relation representations. One purpose of this study is to carry out such an examination in commissurotomized subjects.

Conjoint processing by the disconnected hemispheres

The commissurotomy creates two essentially independent processors within the same brain, and a large part of the research on commissurotomized subjects has been concerned with the separate functioning of the two disconnected hemispheres. There is nonetheless evidence that the disconnected hemispheres are not entirely free from reciprocal interference. For instance, when commissurotomized subjects are required to carry out two tasks simultaneously, one in each hemisphere, each hemisphere operates at a lower level of efficiency than when operating without stimulation of the other (Teng and Sperry, 1973, 1974; Holtzman and Gazzaniga, 1982). This has been interpreted as indicating that the two hemispheres share a common pool of resources that are too limited to accommodate simultaneously the demands of each hemisphere (Holtzman and Gazzaniga, 1982). In addition, when each hemisphere must produce a response of its own after bilateral stimulation, the typical outcome is a response from one hemisphere and extinction in the other, which has been regarded as reflecting a process of 'metacontrol' whereby one hemisphere takes over processing at the expense of the other (e.g., Levy and Trevarthen, 1976; Guiard, 1980). This hemisphere interference suggests some form of rivalry between the two hemispheres taking place at the level of resources and relative activation (e.g., Kinsbourne, 1974) rather than resulting from content- or task-specific conflict, as the two disconnected hemispheres are assumed to perceive and to process information independently of one another.

One aspect of the conjoint functioning of the disconnected hemispheres that has not yet been examined in human commissurotomized subjects is the condition where the two hemispheres are simultaneously presented with the *same* information and must perform the same operation on this information for the production of a *single* response. Such a condition may help elucidate the source of interhemispheric rivalry. If the two hemispheres operate independently of one another, then stimulating each one with the same information for performing the same task should have the same effect as requiring each one to perform a different task, and the factors that affect simultaneous performance on two tasks should also affect performance when the same task is carried out simultaneously in the two disconnected hemispheres. Thus, if performance decrement in the bilateral condition results from a limited amount of resources available to the two hemispheres (e.g., Teng and Sperry, 1973; Holtzman and Gazzaniga, 1982), simultaneous stimulation of the two hemispheres should reduce performance compared with the unilateral condition where only one hemisphere can draw on the full amount of resources. Operationally, this would manifest itself in a lower performance in the bilateral than in the unilateral condition involving the 'specialized' hemisphere.

Alternatively, if the two disconnected hemispheres cannot operate simultaneously and one hemisphere takes control over processing (e.g., Kinsbourne, 1974), performance in the bilateral condition should be as good as, and qualitatively similar to, performance of the more 'specialized' of the two hemispheres. Because these two interpretations assume that the disconnected hemispheres operate in an independent and parallel manner from stimulus reception to response production, they imply that performance in the bilateral condition cannot be better than performance in either unilateral condition. On the other hand, if the two disconnected hemispheres can share the outcome of their respective operations (e.g., Sergent, 1986, 1987), then bilateral performance may be even better than either unilateral performance due to redundant information about the nature of the response to be produced. This, however, would occur only if the two hemispheres are essentially equivalent at performing the required operation and would suggest that the interhemisphere rivalry observed in earlier studies arose from competition at the response production level.

A second purpose of this study is therefore to examine the capacity of the two hemispheres to share the outcome of their respective operations for the production of a single response.

Interhemispheric transfer of visuospatial information

Interest in interhemispheric communication in the commissurotomized brain is now growing, and subcortical transmission has been inferred from the results of visual experiments in which performance of one hemisphere is assessed as a function of the information received by the other. Such transmission has been demonstrated for the presence of large moving stimuli (Trevarthen and Sperry, 1973), the orientation of attention in the visual field (Holtzman, 1984), and semantic and connotative information (Myers and Sperry, 1985; Cronin-Golomb, 1986; Sergent, 1990).

With respect to spatial information, several studies have shown interhemispheric transfer of crude attributes such as location of a large stimulus (Holtzman, 1984) and the orientation of an arrow (Sergent, 1987). Little is known, however, as to how precise this transfer is and what amount of information can be efficiently communicated between the two hemispheres. It is well established that pattern information cannot be transferred as such (Myers and Sperry, 1985; Sergent, 1990), but it is less clear whether this occurs because the patterns used so far were too complex and made of too many components (e.g., common objects, faces) or because subcortical structures cannot represent pattern information whereas they are equipped with structures specialized for the processing of visuospatial information. One question of interest with respect to interhemispheric communication of visuospatial information thus concerns the properties of the information that can be accessed by one hemisphere after stimulation of the other. Is this communication based on categorically coded information to describe location and relative position in space, or does it preserve precise metric information about the position of the object in space?

Another purpose of this study is therefore to examine the nature and limits of interhemispheric communication of visuospatial information in an attempt to specify the 'grain' of this information, and to determine whether the communication takes place in the form of categorical information or preserves metric information.

EXPERIMENTAL INVESTIGATION

This study comprises two main experiments, each composed of several tasks involving lateralized tachistoscopic presentation and the production of a timed single manual response. The first experiment is designed to test the respective competence of the disconnected hemispheres to produce, and operate on, different spatial relation representations, and to examine the effects of requiring the two hemispheres to process the same information conjointly. The second experiment examines the capacity of commissurotomized patients to compare visuospatial information simultaneously projected to each hemisphere, using different tasks to assess interhemispheric exchange of different spatial properties of the stimuli.

General method

Subjects

The subjects were 3 commissurotomized patients, a 55-yr-old woman (N.G.), a 38-yr-old man (A.A.) and a 36-yr-old man (L.B.), who had undergone section of the corpus callosum, the anterior and hippocampal commissures (as well as a large massa intermedia in N.G. and the right fornix in L.B.) in 1963, 1964, and 1965, respectively. Details of their surgical and neurological history have been repeatedly reported (Bogen and Vogel, 1975; Sergent, 1990) and are not described further here. Pictures of their MRI scans have been published by Bogen *et al.* (1988). The subjects were tested in October 1987 and February 1989.

Equipment and stimuli

The stimuli were presented via a Gerbrands tachistoscope and were rear-projected on a translucent screen located 57 cm from the subjects' eyes (such that 1 cm on the screen corresponds to 1° of visual angle). A response panel containing 2 Morse keys was placed in front of the subject. The Morse keys were located along the midline axis, one above the other, and the subjects responded with the index and middle finger of the same hand within an experimental session. Stimulus onset triggered an electronic timer (Gerbrands, Model 130) which was stopped when the subject pressed 1 of the 2 keys. Both speed and accuracy of responses were recorded.

Two types of stimuli were used in this study (*see* fig.). Type 1 (circle-dot) was used in Experiments 1 and 2, and type 2 (circle-spoke) was used in Experiment 2. Stimuli of type 1 were made of a 5 cm diameter circle with a 2 mm thick circumference line, and a 5 mm diameter dot. The dot was located within the circle, in 1 of 9 locations, either along the 45°–225° or the 135°–315° diameter axis, at a distance of either 1 or 2 cm from the centre, or exactly in the centre. Stimuli of type 2 consisted of a circle and a spoke, and 9 such stimuli were constructed, resulting from the orthogonal combination of 3 diameter sizes (3, 4 and 5 cm) and 3 spoke orientations (20°, 45° and 70°, appearing in the upper right quadrant of the circle; *see* fig.).

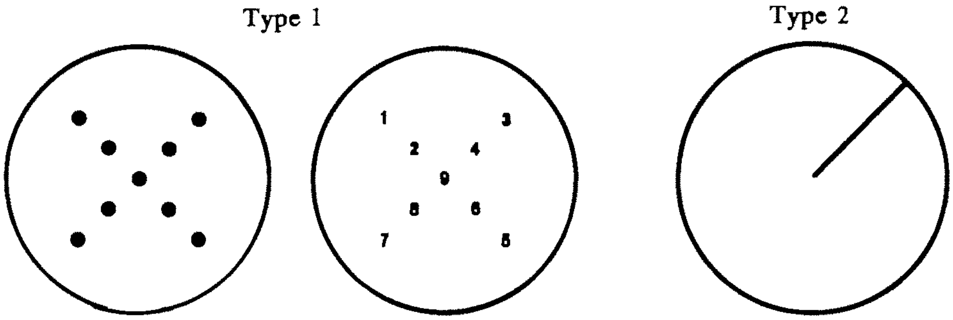

FIG. Representations of the stimuli used in the 2 experiments. *Type 1* ('circle-dot') comprised 9 stimuli each made of a circle and a dot located in one of the 9 positions shown in the left circle; the digits in the right circle correspond to the locations of the dots for reference in the text, *Type 2* ('circle-spoke') comprised 9 stimuli resulting from the combination of 3 circle sizes (3, 4, 5 cm in diameter) and 3 spoke orientations (20°, 45°, 70°).

The stimuli were mounted on slides and appeared as white (87 cd/m^2) on a black background (1.7 cd/m^2), with the centre of the stimulus falling 5° to the right and/or the left of the central fixation point. Exposure duration was 150 ms.

Procedure

Each subject was tested in a dimly lit private room, seated in front of the screen with the head supported by a chin-rest so that the eyes were at the level of the central fixation point. The subjects were requested to hold their legs and feet apart, to keep their teeth tight and their lips closed, and the nonresponding hand lay on the side of the table with no contact with the response panel. A trial started with the signal 'ready' by the experimenter to warn the subject to fixate on the central dot. About 1 s later, a stimulus display appeared, either unilateral or bilateral depending on the task, and the subject was required to make a manual response, according to the instructions, by pressing 1 of the 2 Morse keys. Both speed and accuracy were stressed in the instructions, and the subjects were regularly reminded of the importance of fixating the central point, a procedure with which they are highly familiar. Each task comprised 2 sessions, 1 with each responding hand.

In each task, order of stimulus presentation was quasi random, with no more than 4 trials requiring the same response type in succession. Tasks were first explained to subjects in free viewing, using prints of the stimuli, and then 20 practice trials were given with feedback on response accuracy. The order of responding hand was alternated across tasks and across subjects.

Experiment 1

This experiment involved a series of tasks using unilateral and bilateral presentations of stimuli on which the subjects had to make judgements of spatial relation, either distance or relative position, and it served two main purposes. One was to examine the respective competence of the cerebral hemispheres at representing and judging spatial relations. Kosslyn's theory predicts that the LH should prove superior to the RH at establishing categorical spatial relations and that the RH should be more adept at processing coordinate spatial information. This was examined by requesting the subjects to perform 'categorical' spatial judgements of relative position between a stimulus and a reference point, and to perform judgements of distance between 2 points, using exactly the same stimuli for the two types of judgements. The second purpose was to test the capacity of the two disconnected hemispheres to operate simultaneously, and to determine whether this conjoint processing influences the overall performance. Three possible outcomes can be predicted from current views on this issue. If the two hemispheres must share a limited amount of resources, then bilateral performance will be inferior to unilateral performance; if one hemisphere takes control over processing, then bilateral performance will be quantitatively and qualitatively similar to the better of the unilateral conditions; if the two hemispheres can operate simultaneously, then bilateral performance will be better than either unilateral condition, either because a response can be made whichever hemisphere completes processing first or because the two hemispheres can share the outcome of their respective operations. This was evaluated by comparing the results of the bilateral and unilateral conditions quantitatively, by examining response latencies and accuracies, and qualitatively, by examining the patterns of variations across stimulus conditions.

Methods

The stimuli were the circle-dot patterns shown in the fig. and they were each used in different tasks. There were 9 such stimuli, made of one dot within a circle. Each of the stimuli was presented in 3 visual field conditions: left visual field (LVF), right visual field (RVF), and left and right visual fields (LRVF). Four tasks were carried out on these stimuli.

1. *Centre/off-centre.* A dot could appear in 1 of 9 locations, either in the centre (dot 9, *see* fig.) or off centre (dots 1 – 8). The subjects had to decide whether the dot was exactly in the centre or was off-centre. Two sessions were conducted, each session comprising 2 repetitions of the 8 off-centre dot stimuli in the 3 visual field conditions, and 16 repetitions of the centre dot stimulus, for a total of 96 trials per session.

2. *Near/far.* Only the 8 off-centre dot stimuli were used, and a smaller (2 mm in diameter) dot appeared in the centre of each circle to indicate its location. The subjects had to decide whether the dot was near (dots 2, 4, 6, 8; *see* fig.) or far (dots 1, 3, 5, 7) from centre, and to press the assigned key. Two sessions were conducted, 1 with each hand responding, and a session comprised 4 repetitions of each of the 8 stimuli in the 3 visual field conditions for a total of 96 trials in each session.

3. *Above/below.* Using the 8 off-centre dot stimuli, this task required the subjects to decide whether the dot was above (dots 1, 2, 3, 4) or below (dots 5, 6, 7, 8) the centre, and to press the assigned key, with 96 trials in each of 2 sessions.

4. *Left/right.* This task required the subjects to decide whether the dot was to the left (dots 1, 2, 7, 8) or to the right (dots 3, 4, 5, 6) of the centre, and to press the assigned key. Two sessions of 96 trials were conducted.

A different order of tasks and sessions was used for each subject.

Results

The percentage and the mean reaction times of correct responses of each subject were computed for each task in each session (responding hand) and visual field and are presented in Table 1. The responding-hand factor is included because a preliminary analysis showed that this factor significantly interacted with the visual field.

TABLE 1. MEAN CORRECT RESPONSE LATENCIES (IN ms), AND PERCENTAGE OF CORRECT RESPONSES (BETWEEN PARENTHESES), IN THE 4 TASKS ON THE CIRCLE-DOT STIMULI OF EXPERIMENT 1, AS A FUNCTION OF VISUAL FIELD FOR EACH SUBJECT

		L.B.			*N G.*			*A A.*		
		LVF	*RVF*	*BVF*	*LVF*	*RVF*	*BVF*	*LVF*	*RVF*	*BVF*
Centre/ off centre	LH	653 (81)	660 (78)	651 (97)	691 (81)	759 (75)	743 (91)	930 (66)	1126 (59)	947 (88)
	RH	692 (72)	632 (84)	639 (94)	698 (75)	747 (78)	702 (88)	1023 (66)	1183 (66)	1030 (84)
	Mean	674 (77)	646 (81)	645 (95)	695 (78)	753 (77)	723 (89)	977 (66)	1155 (63)	989 (86)
Near/far	LH	728 (75)	712 (72)	721 (75)	815 (72)	927 (66)	831 (75)	–	–	–
	RH	819 (72)	696 (78)	702 (78)	826 (78)	942 (69)	825 (78)	–	–	–
	Mean	774 (73)	704 (75)	712 (77)	821 (75)	935 (67)	828 (77)			
Above/below	LH	591 (88)	682 (81)	599 (100)	664 (84)	598 (91)	602 (97)	849 (75)	987 (63)	852 (91)
	RH	508 (94)	544 (94)	504 (100)	670 (84)	581 (91)	586 (100)	893 (81)	960 (59)	903 (88)
	Mean	550 (91)	613 (88)	552 (100)	667 (84)	590 (91)	594 (99)	871 (78)	974 (61)	878 (90)
Left/right	LH	554 (81)	627 (63)	582 (94)	603 (75)	642 (69)	611 (91)	784 (75)	952 (59)	882 (81)
	RH	610 (56)	568 (88)	579 (94)	675 (63)	631 (81)	633 (94)	877 (63)	838 (78)	855 (84)
	Mean	582 (69)	598 (76)	581 (94)	639 (69)	637 (75)	622 (93)	831 (69)	895 (69)	869 (83)

LVF = left visual field, RVF = right visual field, BVF = both visual fields, LH = left hand, RH = right hand

Centre/off-centre, near/far, and above/below tasks. These yielded a consistent pattern of results within subjects with respect to LVF and RVF presentations, and they are reported as a function of subjects. The performance of A.A. was at chance in the centre/off-centre task, and in the RVF condition of the above/below task, while he could not follow the instructions during practice in the near/far task. His reaction times were consistently faster in LVF than in RVF presentations when he responded above chance.

N.G. was both faster and more accurate in LVF than RVF presentations in the 2 'distance' tasks (centre/off-centre and near/far), and the difference in reaction times was significant in both tasks (F(1,98) = 5.41, $P < 0.05$; F(1,90) = 6.91, $P < 0.01$, respectively). In contrast, N.G. was faster in RVF than LVF presentations of the above/below task, and the reaction time difference was significant (F(1,111) = 12.15, $P < 0.01$). No visual field difference in accuracy reached a reliable level of significance.

The pattern of visual field performance of L.B. in these 3 tasks was exactly opposite to that of N.G. L.B. was faster and more accurate in RVF than in LVF presentations in the centre/off-centre and the near/far (distance) tasks, and he was faster in LVF than RVF presentations in the above/below task. The differences in response accuracy were not significant but the differences in response latencies were in the near/far task (F(1,94) = 4.48, $P < 0.05$) and in the above/below task (F(1,113) = 5.08, $P < 0.05$).

In none of the subjects and none of the tasks did the bilateral conditions produce faster responses than the faster of the unilateral conditions and, because this was obvious from an examination of the results given in Table 1, no analysis of reaction times was carried out to compare the bilateral and the unilateral conditions. Nonetheless, the bilateral conditions produced reaction times of the same order as the faster of the unilateral conditions. However, response accuracy was higher in the bilateral than in the unilateral conditions, except in the near/far task. These differences never reached significance in the analysis of individual data ($P > 0.05$) but it was reliable in the centre/off-centre and the above/below tasks when considering the data of the 3 subjects together (χ^2 (2) = 11.46 and 7.88, respectively, $P < 0.02$).

Left/right task. This task produced a significant interaction between responding hand and visual field in each subject and for both dependent variables. All 3 subjects responded faster and more accurately to LVF presentations with the left hand and to RVF presentations with the right hand, and this interaction was significant in L.B. and A.A. ($P < 0.05$) but not N.G. ($P = 0.11$).

Because of this interaction the results of the bilateral condition are of particular interest as they allow an examination of whether one hemisphere takes control of processing in the bilateral condition or both hemispheres are operating conjointly. In the 2 subjects (L.B., A.A.) in whom the interaction was significant, the reaction times of the bilateral condition for each hand responding were different from the reaction times of either unilateral condition. This finding was reliable in L.B. for left hand responding ($P < 0.05$) but was marginal for right hand responding ($P < 0.10$) and was not significant in A.A. ($P > 0.20$). The accuracy of response was higher in the bilateral than in either unilateral condition for each responding hand, and this finding was reliable when analysed across subjects (left hand: χ^2 (2) = 12.91, $P < 0.01$; right hand: χ^2 (2) = 14.88, $P < 0.01$).

All these analyses were also carried out in the first half of each session to examine whether the observed effects were present during the early stages of each task. The data showed the same pattern of results as was found in the whole sessions, and, although not all effects reached a reliable level of significance due to the small number of trials on which they were computed, they were in the same direction as in the forementioned analyses.

Discussion

This experiment examined the respective competence of the two disconnected hemispheres at producing, and operating on, representations of spatial relations, and the results showed that both hemispheres have the capacity to represent such spatial relations as all 3 patients performed above chance after LVF and RVF presentations in at least one 'categorical' and one 'coordinate' task. Although this finding departs from predictions based on Kosslyn's (1987) theory postulating that such representations are distinct and mediated by different hemispheres, it is not unexpected. Given the contralateral representation of the visual half spaces in the two hemispheres, each hemisphere must be able to provide cerebral structures underlying spatial cognition with a topographically organized description of the visual world that preserves both relative position and distance, and to perform basic operations on these descriptions. Neither hemisphere has exclusive competence at operating on one type of spatial-relation representation, and evidence from brain-damaged patients indicates that a lesion in either side of the brain is detrimental to the processing of 'coordinate' representations in the contralateral half space (e.g., Brain 1941; Ettlinger *et al.*, 1957; Ratcliff and Davies-Jones, 1972).

The second purpose of this experiment was to examine the effects of conjointly stimulating the two hemispheres with the same information for the production of a single response, in an attempt to understand better the source of hemisphere rivalry observed in earlier studies. One unequivocal finding of this experiment was that the bilateral condition did not result in lower processing efficiency than the unilateral conditions, which should have occurred if the two hemispheres had to vie for a limited amount of resources (Holtzman and Gazzaniga, 1982). There was not one instance of a slower, or less accurate, performance in the bilateral than in either unilateral condition. This contrasts with the situation where the two hemispheres are presented with incompatible information, in which case responses are both slower and less accurate in either unilateral condition and than in bilateral conditions with compatible information presented to the two hemispheres (Sergent, 1983, 1986). In fact, the bilateral condition produced more accurate responses than either unilateral condition in the present experiment. Although the higher accuracy of bilateral than unilateral performance never reached statistical significance in any subject's data analysed separately, it was too systematic across tasks to be a chance finding. Because the unilateral and bilateral conditions involved the same two processors performing the same tasks, a large improvement from the unilateral to the bilateral conditions cannot be expected. Nonetheless, when considered together, the results of the 3 subjects in all but the near/far task showed a reliable increase in response accuracy, and this can be regarded as evidence of a significantly more efficient performance in the bilateral than in the unilateral conditions.

This increased accuracy could not occur unless the two hemispheres simultaneously processed the stimuli and contributed to the overall performance. If only one hemisphere had been processing the stimulus, accuracy could not have been higher in the bilateral condition than in the more accurate of the unilateral conditions.

This finding therefore indicates that, when simultaneously stimulated, the two disconnected hemispheres are able to process the received information separately and in parallel, which does not concur with Kinsbourne's (1974) suggestion that, in the absence of corpus callosum, only one hemisphere can be activated by midbrain structures, nor with Levy and Trevarthen's (1976) proposal of a process of 'metacontrol' whereby one hemisphere would take control of processing. In addition, the finding that accuracy, but not latency, improved in the bilateral condition excludes an interpretation suggesting that only the hemisphere that completes processing first contributed to the response on each trial, and it indicates that a response was the result of processing by the two hemispheres. On the other hand, the present finding does not allow a strong commitment as to the stage during processing at which the two disconnected hemispheres join the outcomes of their respective operations. Two possible stages would be the decision level or the response-production level. This question will be addressed in the General Discussion after examining the capacity of the two disconnected hemispheres to communicate visuospatial information between one another, which is the purpose of the next experiment.

Experiment 2

This experiment was designed to examine interhemispheric comparison of visuospatial information. There is at present some evidence that such information can be transferred between the disconnected hemispheres (Holtzman *et al.*, 1981; Holtzman, 1984; Sergent, 1987), but it is not clear whether this transfer preserves the topographic organization of spatial information or whether it takes place in a form that lends itself to explicit interhemispheric comparison. Holtzman *et al.* (1981) found that cueing one hemisphere with respect to a specific location in the contralateral visual field helped the other hemisphere process a target appearing in the homologous location of the other visual field. However, when an explicit comparison of location between the visual fields was required, one patient (J.W.) performed at chance and the other (P.S.) slightly above chance. In a different type of study, Sergent (1987) found that the patients could decide whether or not an arrow, presented in one visual field, pointed to a dot appearing in the other field but when only considering an arrow presented in the left field, neither patient (L.B., N.G.) could readily tell its orientation. Taken together, these results suggest that visuospatial information *is* transferred, but not in a form that allows explicit localization or description of orientation. The only evidence of transfer indexed by explicit responses was obtained in studies that involved unlimited time for viewing large stimuli moving in the far visual field periphery (Trevarthen and Sperry, 1973) or made of a grid with multiple cues for comparison (Holtzman, 1984). However, the assessment of interhemispheric transfer must not rely uniquely on evidence of explicit or conscious perception or recognition and, especially for visuospatial information which underlies the control of behaviour more or less implicitly, evidence of correct motor response is sufficient indication of interhemispheric transfer (*see* Sergent, 1990, for further discussion). In addition, in as much as visuospatial skills are not restricted to large stimuli, evidence of interhemisphere communication should be sought with relatively small stimuli, and with brief stimulus presentation.

In this experiment, interhemispheric communication of several visuospatial properties was examined and compared with interhemispheric comparison of the stimuli in terms of their identity.

Methods

The 2 types of stimuli shown in the fig. were used in this experiment. In each task, stimulus presentation was bilateral and different pairings of the stimuli were used across tasks, depending on specific spatial attributes, to form two categories of response type.

Series 1. Circle-spoke

The pairing of the 9 circle-spoke stimuli resulted in 45 pairs (36 'different' and 9 'same'). Five tasks were carried out on these pairs of stimuli, each task involving a comparison between the 2 members of a pair appearing 1 in each visual field. In Tasks 1.1, 1.2 and 1.3, 2 sessions were run, 1 with each hand responding.

Task 1.1 involved a comparison as a function of the *size of the circles*, irrespective of the orientation of the spoke. Subjects pressed one key if the 2 circles were of the same size and pressed the other if the 2 circles were of different sizes. Among the 45 pairs, 18 had the 'same' circle size and 27 had 'different' circle sizes, and each was presented twice for a total of 90 trials per session.

Task 1.2 involved a comparison as a function of *spoke orientation*, irrespective of the circle size. Subjects pressed one key if the 2 spokes were of the same orientation, and the other if they were of different orientation. There were 18 'same' pairs and 27 'different' pairs, presented twice in each session.

Task 1.3 involved a comparison as a function of both circle size and spoke orientation, that is, of exact *identity*. Subjects pressed one key if the 2 stimuli were the same and the other if they were different. Each of the 9 'same' pairs was repeated 4 times, and the 36 'different' pairs were used. They were presented twice in a session for a total of 144 trials in each session.

Task 1.4 was similar to Task 1.1, with two exceptions. The response panel was rotated 90° such that the keys were positioned side by side horizontally, and the subjects responded with both hands, one hand for each key. The subjects were required to press the key on the *side of the larger circle*, and to refrain from responding if the 2 circles had the same size. Each of the 9 'same' pairs appeared twice which, along with the 9 'different' pairs having circles of the same size, resulted in 27 'same size' circle pairs, and 27 'different size' circle pairs. They were repeated twice and 1 session of 108 trials was run.

Task 1.5 used the same pairing of stimuli as Task 1.2 and the same procedure as Task 1.4. The subjects were required to press on the key corresponding to the side in which the stimulus with *the spoke oriented the closer to the vertical* appeared, and to refrain from responding if the spoke had the same orientation in each circle. There were 27 pairs with the spoke in the same orientation and 27 pairs with the spoke in different orientations, and they were repeated twice in one session of 108 trials.

Although the subjects were timed in each task to ensure quick response and prevent them from resorting to some cross-cueing strategy, the dependent variables of interest in this experiment was response accuracy To avoid a too lengthy report of the results, only percentages of errors are reported here. Response latencies averaged 900 ms for L.B. and N.G., and 1400 ms for A.A.

Results

The results are shown in Table 2, in terms of percentage of correct responses in each task as a function of response type for each of the 3 subjects. A preliminary analysis showed no significant effect of the responding hand on response accuracy, and the results have been averaged over this factor.

An examination of Table 2 indicates variations across tasks and across subjects in the capacity of the commissurotomized brain to compare visuospatial information divided between the two hemispheres. The only task that all 3 subjects failed was the comparison of the stimuli as a function of both circle size and spoke orientation, that is, exact identity (Task 1.3: χ^2 (1) < 1 in all conditions and subjects). In contrast, both L.B. and N.G. were able to perform above chance when comparing the stimuli as a function of circle size (Task 1.1: L.B.: χ^2 (1) $= 10.68$, $P < 0.001$; N.G.: χ^2 (1) $= 41.34$, $P < 0.001$), whereas A.A.'s responses were not significantly different from chance ($\chi^2 < 1$). When comparing the stimuli in terms of spoke orientation (Task 1.2), N.G. performed significantly above chance (χ^2 (1) $= 33.61$, $P < 0.001$), and L.B. and A.A. responded at chance although, when considering the total number of trials for L.B., his performance (110/180) was slightly above chance (χ^2 (1) $= 4.44$, $P < 0.05$) but was at chance in both response-type conditions.

Tasks 1.4 and 1.5 required a different mode of response as the subjects had to indicate the visual field in which the larger circle, or the spoke the closer to the vertical, appeared, and to refrain from responding if the relevant attribute was the same in the 2 circles. These 2 tasks resulted in high accuracy levels, and all 3 subjects performed above chance when responding as a function of circle size (Task 1.4: L.B.: χ^2 (1) $= 25.04$, $P < 0.001$; N.G.: χ^2 (1) $= 23.15$, $P < 0.001$; A.A.: χ^2 (1) $= 12$, $P < 0.001$); L.B. and N.G. were also performing above chance when responding as a function of spoke orientation (Task 1.5: χ^2 (1) > 15, $P < 0.001$ for these 2 subjects) as did A.A. in one condition (when the correct response was on the right field) but not in the other condition (χ^2 (1) $= 2.24$, $P > 0.10$). The percentage of false alarms (pressing 1 key when the relevant attribute was the same in the 2 circles) was less than 10% for L.B. and N.G., and less than 25% for A.A. in both tasks.

The subjects showed no marked difference in behaviour whether they responded at or above chance, except L.B. in Task 1.3 (identity comparison) where he was aware that he was not performing well. Although Task 1.1 was conceived as a comparison of circle size, L.B. mentioned after the task that he based his decisions on the height of the circles rather than their size; neither N.G. nor A.A. could explicitly describe the strategy they were using in this task.

TABLE 2 PERCENTAGE OF CORRECT RESPONSES IN THE 5 TASKS ON THE CIRCLE-SPOKE STIMULI FOR THE 3 PATIENTS

	L.B.	*N.G*	*A.A*
Task 1.1. Circle size			
Same (n = 72)	72.2	86.1	45.8*
Different (n = 108)	63.9	82.4	52.8*
Total (n = 180)	67.2	83.8	50.0*
Task 1.2. Spoke orientation			
Same (n = 72)	65.3*	83.3	55.6*
Different (n = 108)	58.3*	78.7	49.1*
Total (n = 180)	61.1	80.6	51.7*
Task 1.3. Identity			
Same (n = 144)	52.8*	55.6*	44.4*
Different (n = 144)	55.6*	48.6*	52.8*
Total (n = 288)	54.2*	52.1*	48.6*
Task 1.4. Side of larger circle			
Larger on left (n = 27)	96.3	100.0	85.2
Larger on right (n = 27)	100.0	92.6	81.5
Total (n = 54)	98.1	96.3	83.3
Task 1.5 Closer to vertical			
Closer on left (n = 27)	92.6	92.6	70.4*
Closer on right (n = 27)	96.3	85.2	77.8
Total (n = 54)	94.5	88.9	74.1

* Not significantly different from chance

Series 2. Circle-dot

There were 9 stimuli of this type made of a circle and a dot (*see* fig.), from which 36 'different' pairs and 9 'same' pairs were obtained Five tasks were conducted, and 2 sessions were run in each task, 1 with each hand responding.

Task 2.1 involved a comparison as to whether the two dots were exactly *in the centre* or *off-centre*. The subjects pressed one key if they were in the centre and the other key if they were off-centre. There were 36 pairs with an 'off-centre' dot and the 'centre-dot' pair was presented 30 times, making a total of 66 trials. The same number of trials was included in which a pair comprised an 'off-centre' dot in one field and a 'centre dot' in the other, and the subjects had to refrain from responding to such pairs.

Task 2.2 involved a comparison as a function of the *near or far* distance of the dots from the centre. Only stimuli with an 'off-centre' dot were used. Subjects pressed one key if the 2 dots were near the centre (dots 2, 4, 6, 8; *see* fig.) and they pressed the other key if they were far from the centre (dots 1, 3, 5, 7). There were 10 pairs in each category, repeated 3 times for 60 trials. The same number of trials was included in which a pair comprised a 'far' dot in one field and a 'near' dot in the other, and the subjects had to refrain from responding.

Task 2.3 involved a comparison as a function of *latitudinal location* of the dot in the circle, using only stimuli with an 'off-centre' dot. If the 2 dots were on the same side of the circle (both above or both below the centre), the subjects pressed one key, and the other if the dots were on opposite sides of the centre. There were 20 'same' pairs and 16 'opposite' pairs, presented 3 times in each session.

Task 2.4 involved a comparison as a function of *longitudinal location* of the dot in the circle, using only stimuli with an 'off-centre' dot. If the dots were on the same side of the circle (both to the right or both to the left of the centre), the subjects pressed one key and the other if the 2 dots were on opposite sides of the centre. There were 20 'same' pairs and 16 'opposite' pairs, presented 3 times in each session.

Task 2.5 involved a comparison as a function of exact *identity*. The subjects pressed one key if the 2 dots were exactly in the same location, and they pressed the other if they were in different locations. There were 36 'different' pairs and an equal number of 'same' pairs were used by repeating each 'same' pair 4 times. These stimuli were presented twice in a session. The same procedure as in the earlier tasks was used. When only stimuli with off-centre dots were presented in a task, a small (2 mm in diameter) central dot was present in each circle to indicate the location of the centre.

Results

The results are shown in Table 3, in terms of percentage of correct responses averaged over the responding hands, in each task as a function of response type for each of the 3 subjects.

TABLE 3 PERCENTAGE OF CORRECT RESPONSES IN THE 5 TASKS ON THE CIRCLE-DOT STIMULI FOR THE 3 PATIENTS

	L.B.	*N.G.*	*A.A.*
Task 2.1. Centre/off-centre			
Centre (n = 72)	76.4	66.7	54.2*
Off-centre (n = 60)	80.0	73.3	45.0*
Total (n = 132)	78.0	69.7	50.0*
Task 2.2. Near/far			
Near (n = 60)	45.0*	–	–
Far (n = 60)	48.3*	–	–
Total (n = 120)	46.7*	–	–
Task 2.3. Above/below			
Same side (n = 80)	86.3	90.0	70.0
Opposite sides (n = 64)	75.0	81.3	71.9
Total (n = 144)	81.3	86.1	70.8
Task 2.4. Left/right			
Same side (n = 80)	78.8	71.3	53.8*
Opposite sides (n = 64)	64.1*	60.9*	56.3*
Total (n = 144)	72.2	66.7	54.9*
Task 2.5. Identity			
Same (n = 72)	56.9*	45.8*	51.4*
Different (n = 72)	51.4*	48.6*	43.1*
Total (n = 144)	54.2*	47.2*	47.2*

* Not significantly different from chance.

The 3 patients failed the identity task (Task 2.5: χ^2 (1) < 1 in all conditions and subjects), and both L.B. and N.G. failed the near/far distance task (Task 2.2: L.B.: χ^2 (1) < 1; N.G. did not follow the instructions during the practice trials; A.A. was not tested on this task). The only task in which all 3 subjects performed above chance was Task 2.3, when they decided whether the dots were on the same (above or below) or opposite sides of the centre (L.B.: χ^2 (1) $= 28.13$, $P < 0.001$; N.G. χ^2 (1) $= 37.56$, $P < 0.001$; A.A.: χ^2 (1) $= 12.5$, $P < 0.001$). Both L.B. and N.G. performed above chance when deciding whether the 2 dots were on or off centre (Task 2.1: χ^2 (1) > 8, $P < 0.01$, in all conditions). The performance of these 2 subjects yielded mixed results in Task 2.4 where they had to decide whether the 2 dots were on the same (left or right) or opposite sides of the centre. They responded above chance to the 2 dots on the same side (L.B.: χ^2 (1) 13.23, $P < 0.001$; N.G.: χ^2 (1) $= 7.23$, $P < 0.01$), but not when they were on opposite sides (L.B.: χ^2 (1) $= 2.53$, $P > 0.05$; N.G.: χ^2 (1) $= 1.53$, $P > 0.10$). The performance of A.A. on this task was not significantly different from chance.

An additional analysis was carried out to examine the effects of dot location on the decision that 2 dots were on the same side. The trials of the 'same side' condition of Tasks 2.3 and 2.4 were divided into 'exactly the same location' of the 2 dots (32 out of 80 trials) and 'different locations' of the 2 dots within the same side (48 out of 80 trials) to examine the effects of dot location on the accuracy of response. The 3 subjects responded more accurately when the 2 dots were in the same than in different locations within a side in the 2 tasks, and these results are shown in Table 4. An examination of this table indicates that response accuracy was higher in the 'exactly the same location' condition of Tasks 2.3 and 2.4 than in the corresponding 'same' condition of Task 2.5, and this difference was statistically reliable ($P < 0.01$).

TABLE 4 PERCENTAGE OF CORRECT RESPONSES IN THE 'SAME SIDE' CONDITION OF TASKS 2.3 AND 2.4 AS A FUNCTION OF 'EXACTLY THE SAME LOCATION' AND 'DIFFERENT LOCATIONS', AND IN THE 'SAME' CONDITION OF TASK 2.5

	L.B.	*N.G.*	*A.A.*
Task 2.3			
'Exactly the same location'	90.6	96.8	87.5
'Different locations'	83.3	85.4	58.3
Task 2.4			
'Exactly the same location'	90.6	84.4	75.0
'Different locations'	70.8	62.5	43.8
Task 2.5			
'Same'	56.9	45.8	51.4

Discussion

In this experiment, 3 commissurotomized subjects performed a series of tasks aimed at examining the nature, the extent, and the 'grain' of the interhemispheric communication of visuospatial information. The results extend earlier findings (Holtzman, 1984; Sergent, 1987) in showing that the disconnected hemispheres are able to establish a relation between spatial locations in the two visual fields, and provide new indications about the nature of this relation and some of its underlying operations.

With each stimulus type, performance was systematically better when the comparison could be achieved in terms of categorically defined space. This was typically the case in the above/below, and the left/right, comparisons. This, however, does not seem to reflect a categorical encoding of the visuospatial information before interhemispheric comparison. For one thing, the better performance on these tasks may have resulted from the less stringent demands on the precision with which spatial information had to be encoded, as any dot within a half-space qualified as belonging to a given category. Moreover, if the comparison were taking place after recoding of the information into a category, there should be no difference in response accuracy as a function of the location of the dot within a given half-space, as a category is not bound to any location. Yet the results clearly showed that subjects were more accurate when the 2 dots were in exactly the same location within the circles than when they were not, and this suggests that the information on which the comparison was performed contained precise specification of the actual location of the dots within their respective space Three implications may follow from these results. First, metric information is preserved in the spatial representations on which interhemisphere comparison is performed. Secondly, if the two disconnected hemispheres can compare their respective information on the basis of categorical and metric information, it implies that both hemispheres are capable of producing the two types of spatial relation representations. Thirdly, the finding that relatively precise location of a dot can be communicated between the two disconnected hemispheres contrasts with the well established evidence that pattern information representing visual objects cannot be transferred between the hemispheres in the commissurotomized brain, even more than 20 years after surgery (Bogen, 1985; Sergent, 1990).

One reason for this difference in interhemispheric transfer between visuospatial and pattern stimuli may lie in the different amount of information that characterizes each category. As shown in the present experiment, interhemisphere comparison was successful when the relevant attributes varied along a single dimension but failed when two dimensions had to be considered conjointly (e.g., Task 2.3 as opposed to Task 2.5). In addition, N.G. was able to compare the circle-spoke stimuli as a function of each separate attribute (Tasks 1.1, 1.2) but not of the two attributes together (Task 1.3). In as much as visuospatial information does not vary along as many dimensions as do pattern stimuli, interhemispheric communication may be more easily achieved with the former than with the latter. This explanation may not be sufficient, however, to account for all differences between the two categories, as interhemisphere transfer fails even with simple pattern stimuli such as digits (Sergent, 1990).

A second reason for the difference between the two categories may then come from the different structural organization of the neural substrates underlying their respective processing. Meaningful pattern information is readily translated into semantic codes that are necessary for further operation on this information, and there is evidence that, when interhemisphere communication of information about objects takes place, it is in terms of such semantic codes rather than their physical attributes (Myers and Sperry, 1985; Sergent, 1990). After section of the neocortical commissures, detailed visual information is confined to the hemisphere that receives it and only if this information is translated into semantic codes does some interhemisphere communication take place. In contrast, visuospatial information processing, to serve behaviour, must be performed on topographically organized representations in order to inform the processing structures about the location, relative position, and distance of the stimulus in space, and a translation of this information into some category would result in a loss of critical parameters for guiding spatial behaviour. Such a coordinate representation of the visual world is found not only in cortical structures but also in subcortical structures such as the superior colliculus (Cusick, 1988; Stein, 1988). This structure may then contribute to interhemispheric communication through its own commissures as long as the resolving demands and the complexity of the stimuli are not too high (Sparks and Jay, 1987). Representations of visual objects, even if they are intrinsically spatial, also contain intercomponent relationships and configurational properties that may be beyond the encoding capacity of the superior colliculus, whereas an accurate mapping of visuospatial information, described along vertical and horizontal axes, may be achieved in this structure.

The foregoing considerations could suggest that the interhemispheric comparison observed in this experiment did not require the involvement of cortical structures and could be achieved entirely through subcortical processing, but there is reason to believe that this is not so. As shown by Holtzman (1984) and Sergent (1988), the performance of commissurotomized and hemianopic subjects is qualitatively different in tasks that require transfer of visuospatial information. If the superior colliculus alone was sufficient to subserve the encoding, processing, and transfer of information presented in one visual field, hemianopic subjects, in whom such subcortical structures are intact, should be able to achieve a comparison of bilaterally presented information, but they are not (Holtzman, 1984). Thus an account of the present results must involve a close interaction between cortical and collicular structures, and both are necessary for an efficient interhemisphere communication of visuospatial information in the absence of neocortical commissures. It is an empirical question to determine whether this also applies to patients born without a corpus callosum.

Two further aspects of the findings deserve consideration. One concerns the different patterns of results in the comparison of attributes of the circle-spoke stimuli depending on the experimental condition in which interhemisphere communication was assessed. Tasks 1.1 and 1.2 required a 'same-different' judgement between one relevant attribute of each circle (circle size and spoke orientation), and A.A. performed the 2 tasks at chance and L.B.'s performance was at chance in Task 1.2. On the other hand, Tasks 1.4 and 1.5 also involved a comparison between the same separate attributes, but they required a decision as to which of the 2 stimuli had the larger circle or had the spoke the closer to the vertical. With such instructions, both A.A. and L.B. improved their performance in the comparison of the same attributes that they had not reliably compared in the 'same-different' task. The main difference between the two types of instructions is that, while one type provides the subjects with no indication as to how to compare the relevant attributes, the other gives the subjects a clue about a possible way of encoding, and perhaps categorizing, them for a more efficient comparison (such a categorization may have been possible in this particular task because only 3 variations could occur within a dimension). That this may have been the case is further suggested by the results for N.G. who was more accurate with the latter than the former type of instructions. The different outcomes of the comparison of the same visual attributes depending on the type of instructions illustrate the importance of task demands in the evaluation of communication between the two disconnected hemispheres.

A second aspect of the findings worthy of discussion is the different level of accuracy in response to the same 2 dots depending on the nature of the comparison. For instance, in Tasks 2.3 and 2.4, the subjects compared 2 dots appearing in exactly the same location with high accuracy when they were instructed to respond as a function of the side of the dot; in contrast, in Task 2.5, they were unable to make a reliable comparison between the same 2 dots when the instructions specifically called for attending to the exact location of the dots. On the basis of the results of Task 2.5, the conclusion would be that an efficient relation between homologous position in the two lateral visual fields cannot be achieved in the commissurotomized brain, and that one hemisphere does not know the exact location of a dot in the ipsilateral field. Yet the results of Tasks 2.3 and 2.4, in which the exact location of a dot was not a relevant factor in the comparison, showed that interhemisphere comparison was influenced by this factor, suggesting that information about the exact location of the dot had been encoded and could be put in relation between the two hemispheres. There is therefore a dissociation between the implicit knowledge of spatial location and the use of this knowledge in tasks that explicitly require its processing. This finding extends Holtzman's (1984) demonstration of a dissociation between interhemispheric priming and comparison in a spatial task and it provides yet another illustration of implicit processing of information that cannot be brought to awareness (Schacter, 1990).

GENERAL DISCUSSION

This study examined the processing of spatial relations within and between the disconnected hemispheres, and it provides new information about the operations underlying these processes and the functioning of the commissurotomized brain.

Hemisphere processing of spatial relations

In spite of the well established critical role of the RH in spatial processing, evidenced in studies of brain-damaged patients (De Renzi, 1982) as well as of the 3 patients tested here (e.g., Franco and Sperry, 1974; Sergent and Corballis, 1989), the results offered no indication that representations of categorical and coordinate spatial relations would be produced by different hemispheres, as proposed by Kosslyn (1987). Instead, they indicated a functional hemisphere equivalence, suggested by the capacity of each disconnected hemisphere to make judgement of distance and relative position, by the absence of consistent hemisphere asymmetry across subjects, by the enhanced accuracy in the bilateral condition implying that both hemispheres can implement the required operations, and by the ability of the two disconnected hemispheres to compare their respective respresentations of spatial relations.

Although inferences from commissurotomized patients about the normal brain require caution, it is noteworthy that these patients showed a clear RH superiority in the processing of faces and spatial transformations when tested at about the same time (Sergent and Corballis, 1989; Sergent, 1990). If a hemisphere dissociation between the representations of the two types of spatial relations were a basic property of the brain, it should at least manifest itself in different levels of processing efficiency in the same way that it has reliably manifested itself with other cognitive functions in these patients. Thus when considering the perceptual processing of spatial relations as such, independent of attentional and memory functions that may engage structures of the RH more than those of the LH, there seems to be no reason to expect a functional hemisphere asymmetry, as these operations must be performed on topographically organized representations (e.g., Ratcliff and Davies-Jones, 1972).

It is noteworthy, nonetheless, that the pattern of results displayed by N.G. in Experiment 1 tended to conform to the predictions based on Kosslyn's (1987) suggestions, but the results of the other 2 patients failed to support these predictions. This diversity of findings with respect to the processing of spatial relations also characterizes research on normal and brain-damaged subjects. For instance, Luria (1973) reported cases of left parieto-occipital patients able to draw geometric shapes on instruction but unable to place one shape above or below another when so requested, and Brain (1941) and Paterson and Zangwill (1944) reported similar deficits in patients suffering from right parietal damage. Similarly, impaired judgements of distance and localization are often more pronounced after right than left posterior damage, which could point to a critical role of the RH in operations performed on coordinate spatial representations (e.g., De Renzi, 1982), yet deficits in such tasks are systematically more pronounced in the right visual field of LH than RH-injured patients (e.g., Tartaglione *et al.*, 1981). It may then appear that the diversity of results is such as to support almost any hypothesis by the manner in which relevant findings are selected, and a conservative conclusion is that both hemispheres are essential to the processing of spatial relations in their respective visual space.

Conjoint processing by the disconnected hemispheres

The bilateral stimulus presentations provided an opportunity to examine the conjoint functioning of the disconnected hemispheres and to evaluate three current views regarding this functioning. No support was found for the view that, because the two disconnected hemispheres must draw on a limited amount of resources (Holtman and Gazzaniga, 1982), bilateral performance should be reduced compared with the better of the unilateral conditions. There was no support, either, for the view that only one hemisphere can operate at a time and takes control of processing (Kinsbourne, 1974; Levy and Trevarthen, 1976; Guiard, 1980). The fact that Experiment 2 yielded above-chance performance by itself suggests that the two disconnected hemispheres can operate simultaneously. In addition, the enhanced response accuracy obtained in the bilateral conditions of Experiment 1 compared with the unilateral conditions implies a contribution of the two hemispheres to the overall performance.

Yet earlier findings had unequivocally shown that bilateral presentations resulted in reduced performance in each hemisphere compared with their performance when operating without concurrent involvement of the other (e.g., Teng and Sperry, 1974), and a typical outcome was that only one hemisphere produced a response after bilateral stimulation. A process of ‘metacontrol’, and ‘perceptual erasure’ in one hemisphere have been suggested to account for the neglect of one visual field (Levy and Trevarthen, 1976; Guiard, 1980), but this is an unlikely explanation of the phenomenon, and an experiment by Levy *et al.* (1972) may offer an illustration. These authors found that, after bilateral stimulation with chimeric stimuli, and when a spontaneous response from one hemisphere was blocked and a response was requested from the other hemisphere, the latter hemisphere could produce a response based on the information it had received. Both hemispheres had in fact effectively processed their respective information, and the source of the interference was at the response-production level. By requesting a single response after bilateral stimulation in the present study, no interference occurred, and improved performance prevailed in most conditions. This finding is inconsistent with the view that the two disconnected hemispheres cannot operate stimultaneously, and instead suggests that they have difficulty producing two different responses simultaneously, presumably because of the convergence of the two hemispheres towards the same neural structures underlying the preparation and organization of a motor response.

Further evidence that both hemispheres contributed to the final responses comes from the results of the left/right task of Experiment 1. Because of a spatial compatibility effect, the subjects responded faster with the left hand to the left field and with the right hand to the right field in the unilateral conditions. However, the bilateral condition yielded intermediate latencies unlike those of either unilateral condition. The two hemispheres must therefore have contributed their respective input to the response process and, despite the initial independent processing of the information in the two hemispheres, the commissurotomized brain responded as a function of the two visual fields, as is typically found in subjects with intact commissures (Sergent, 1991). These findings add to the evidence that the disconnection of the two cortices does not prevent the commissurotomized brain from acting as a single organ (Sergent, 1987).

Interhemispheric communication of visuospatial information

The communication of visuospatial information between the two disconnected hemispheres was found to be of a nature different from that of pattern information. Whereas the latter is not communicated as such, but in terms of semantically processed information, comparison of visuospatial representations between the two hemispheres could be achieved as a function of relatively precise information about location in space. This reflects the different physical composition and substrates of the two types of information, and suggests that subcortical structures, presumably the superior colliculus, are equipped to preserve the coordinate representations of visuospatial information and may serve as processing relays for establishing relationships between the two visual fields. Even if this interhemispheric exchange does not eliminate the topographic organization of spatial information, it nonetheless seems to be restricted to a limited amount of information. Efficient comparison of stimuli that varied along two dimensions could not be achieved even though each of these dimensions could be compared with a high level of accuracy.

However, this failure to compare stimuli varying along two dimensions contrasts with the subjects' capacity to perform comparisons of information in the above/below task of Experiment 2 with a precision that could be obtained only if the location of the dots on the two dimensions had been encoded and put in relation between the two hemispheres. There was, therefore, more information accessible from the contralateral hemisphere than a direct comparison between informational contents explicitly held by each hemisphere would suggest. This finding has important implications for the understanding of the callosal disconnection syndrome. This syndrome, which is characterized by a failure of communication between the two sides of the brain, is considered to reflect deficits due to a physical interruption of interhemispheric pathways. However, part of this syndrome may result from an inability of the hemispheres to become aware of some effectively transferred information. As shown in the results on the circle-spoke stimuli, all tasks are not equally sensitive to uncover the occurrence of interhemispheric transfer. Moreover, as shown in the results on the circle-dot stimuli, transferred information may influence performance in a way that manifests itself only when the subjects are not explicitly instructed to attend to it but nonetheless make use of it unintentionally. It may then be that each disconnected hemisphere is better informed about the operations and informational contents of the other than each can explicitly indicate, and it is left for further investigation to determine the extent and the nature of the information the two disconnected hemispheres implicitly share.

ACKNOWLEDGEMENTS

This research was supported by the Medical Research Council of Canada and the National Institute of Mental Health. I thank Professor Roger Sperry for allowing me to use his laboratory facilities and to test the patients, Dr Charles Hamilton for his help and assistance with the arrangements, and the patients for their untiring cooperation. I also acknowledge the perceptive comments of two anonymous reviewers on an earlier version of this article.

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(Received January 16, 1990. Revised June 13, 1990. Accepted June 26, 1990)

Brain (1991), **114**, 1045–1056

THE NATURAL HISTORY OF MULTIPLE SCLEROSIS: A GEOGRAPHICALLY BASED STUDY

3. MULTIVARIATE ANALYSIS OF PREDICTIVE FACTORS AND MODELS OF OUTCOME

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SUMMARY

A multivariate hierarchical analysis was used to assess the significance of several demographic and clinical factors in multiple sclerosis patients. We used the time to reach level 6 on the disability status scale (DSS) of Kurtzke as endpoint. Several factors at presentation were significantly associated with an adverse outcome including older age at onset, male sex, cerebellar involvement or insidious onset of a motor deficit as first symptom. Factors ascertained later which were associated significantly with a worse outcome, even after controlling for those previously mentioned, included persisting deficits in brainstem, cerebellar or cerebral systems, a higher frequency of attacks in the first 2 yrs after onset of disease, a short first interattack interval and higher DSS at 2 yrs and 5 yrs from onset. An analysis similar to multiple regression was used to generate predictive models which permit the calculation of the median time to DSS 6 for patients with a given set of covariates. The goodness of fit of these models to the data and their predictive accuracy are discussed.

INTRODUCTION

In conducting randomized controlled clinical trials, it is sometimes advisable to generate homogeneous groups of patients by stratification for variables associated with outcome. Multiple sclerosis (MS) is a complex disease, the clinical manifestations and course of which vary considerably. Nonetheless, patients are often selected on the basis of a single inclusion criterion. In trials of immunosuppressive drugs in MS, a progressive course is the criterion most commonly used; it is widely accepted as the best predictor of adverse outcome (Weinshenker and Ebers, 1987). However, failure of randomization of patients to treatment groups to control for the effects of other variables results in extraneous unexplained variation in observed outcome among randomized subgroups in a clinical trial, resulting in loss of power. Furthermore, clinicians often determine that progressive disease has intervened after moderate and perhaps fixed disability is present. The presence of fixed disability limits the usefulness of disability assessments in therapeutic trials of agents which potentially lessen the activity of MS. It is possible that therapies may become available which are both effective and associated with less morbidity. Accordingly, it would be desirable to identify patients at an earlier point in

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their disease having one or more parameters known to be associated with an adverse outcome but not reflecting the presence of irreversible disability.

We have previously described our results of the natural history of MS in terms of a failure time analysis using several points on the Kurtzke disability status scale (DSS) as endpoint (Weinshenker *et al.*, 1989*a*). These data were based on serial follow-up of a clinic-based population of 1099 MS patients followed longitudinally between 1972 and 1984 at the MS Clinic, University Hospital, London, Canada. In a second report, we have shown that a number of parameters reflecting the early course of MS (number of attacks in the first 2 yrs after onset of MS, the first interattack interval, time to reach DSS 3) influenced clinical outcome measured by survival analysis using DSS 6 (aids required for ambulation) as endpoint (Weinshenker *et al.*, 1989*b*). However, stratification for each variable independently left considerable unexplained variation in the time to reach DSS 6. A multivariate hierarchical analysis is utilized in this report using an accelerated failure time model to assess the significance of several variables associated with outcome. The approach allows one to evaluate the independent contribution of prognostic indicators by holding the effect of others constant. The parameters are tested in a hierarchical way according to the sequence in which they are revealed in a clinical setting.

METHODS

The London MS Clinic and data collection

The data base has been previously described (Weinshenker *et al.*, 1989*a*) Briefly, the data are based on follow-up between 1972 and 1984 of 1099 patients primarily from southwestern Ontario who were evaluated on an annual basis regardless of clinical outcome. Of these patients. 197 presented for a neurological opinion at the time of their first symptom, and documentation of disability and course of the disease was present from onset. Charts were reviewed and disability was ascertained from standard data forms recorded at each patient visit. Key levels of disability, for which there is relatively close agreement among different evaluators, were chosen as endpoints. Interobserver variation has been assessed prospectively in a Canadian multicentre clinical trial and is generally small within 1 level of the DSS (Noseworthy *et al.*, 1988) Furthermore, the longitudinal nature of the follow-up mitigates the problem of minor fluctuation in disability scores unrelated to fixed disability Other parameters were recorded as previously described (Weinshenker *et al.*, 1989*a*).

Statistical analysis

Survival (failure to reach DSS 6) is taken to be a function of time and hazard (the probability per unit time that cases entering the interval under consideration will reach DSS 6) Survival analysis allows for right censoring of cases, that is, it considers both patients who have and have not reached the endpoint at each point in time. The simple exponential distribution of survival analysis assumes hazard to be a constant, but this can be generalized to allow for a dependence of hazard on time by invoking the Weibull distribution (Kalbfleisch and Prentice, 1980). A random baseline of survival independent of covariates was assumed to follow the Weibull distribution. Covariates were assumed either to accelerate or decelerate the attainment of the endpoint, DSS 6, in a multiplicative way To determine if the Weibull model adequately represented the data, plots of the log (−log (survival)) vs log (time) were generated. A straight line plot indicated the appropriateness of the assumption.

Initially, we attempted to determine which demographic and clinical factors were associated with survival. A series of covariates were considered in a hierarchical way; in this context hierarchical refers to the sequence in which data on these covariates would become available in the evaluation and follow-up of MS patients. Initially we considered demographic data, followed by initial symptoms, clinical course, neurological systems involved and finally details of the early course of the disease. The statistical significance of each covariate in the survival model was tested by a χ^2 statistic with one degree of freedom. After testing factors related to patient demographics, other factors were tested sequentially one at a time, controlling for the effects of covariates previously found to be significant at the $P < 0.05$ level. Because of association of various covariates with one another, factors previously significant became insignificant as newer more definitive information regarding the clinical course became available.

To obtain predictive models which describe the median time to reach DSS 6, a multiple regression analysis was used, based on the LIFEREG procedure in the SAS statistical package (SAS Institute Inc SAS users guide: Statistics, version 5 edition, Cary, NC, 1985). This allows for right censoring of cases and for specification of the random baseline (Weibull distribution). A series of models was generated again using the same hierarchical approach. In each model the log (time DSS 6) is given by the sum of the products of the regression coefficients and their respective parameters and a correction factor for the Weibull distribution The relative magnitude of the absolute value of the coefficients for binary parameters give a quantitative picture of their influence on outcome. These models were generated to maximize the log of likelihood which is a measure of the likelihood that the observed data would be generated by the model. This is dependent on sample size and in successive models there was a fall off in the number of patients with available data. Finally, the distribution of errors of prediction were examined for various models so as to demonstrate graphically how well the models predict the observed outcome. The predictive advantage of multivariate models was considered by applying 3 of the models to the same set of patients with data available for each of the 3 models The reduction of prediction error in successive models over that from knowledge of the median time to DSS 6 is a measure of predictive advantage.

RESULTS

Tests of significance of factors associated with outcome

Table 1 shows the coefficients of the hierarchical parameters tested and their level of significance as to whether they influence the time to reach DSS 6. A negative coefficient implies an adverse influence on outcome (i.e., shortening of time to DSS 6). Male sex, older age at onset of MS, and being seen from onset of MS were all significantly associated with an adverse outcome. Controlling for these factors, the presence of optic neuritis at onset was associated with a favourable outcome ($P < 0.03$). Slow onset of a motor deficit, and limb ataxia or impairment of balance suggesting cerebellar involvement, were both associated with an adverse outcome. Acute onset of a motor deficit was not significantly associated with outcome; this was a presenting symptom in only 6.2% of cases. As expected, progressive disease from onset (either relapsing progressive or chronically progressive) was associated significantly with an adverse outcome. When major involvement of neurological systems at final review was considered, those most significantly associated with an adverse outcome were cerebellar involvement (15.8% of patients) and cerebral involvement (7.0%). Brainstem involvement (37.5%) was also significant. Pyramidal involvement (79%) was not found to be significantly associated with the rate at which DSS 6 was reached even though pyramidal involvement is heavily weighted in the scoring of the DSS. Even when controlling for factors previously found to be significant, parameters reflecting the early course of MS were highly significant in predicting time to DSS 6 (Table 1F). While the presence of oligoclonal IgG bands in CSF and DR2 positivity on HLA typing were both marginally associated with adverse outcome, this may be due in large part to the selective ascertainment of these results. Patients attending clinic more frequently, generally those with the most active disease, were more likely to have had these tests performed. Results of HLA typing were available in only 288 patients and results of CSF electrophoresis were available in only 527 cases.

Several associations among covariates were found to be significant during this analysis. Cerebellar involvement was seen in 21.8% of males as compared with 12.9% of females

TABLE 1 HIERARCHICAL TESTS OF SIGNIFICANCE

	Coefficient	*P value*
A Demographic characteristics		
Age at onset	−0 030	<0 001
Male sex	−0 175	0.004
Seen at onset of MS	−0.499	<0.001
B Initial symptoms (controlling for age at onset, seen at onset and sex)		
Optic neuritis	0.182	0.03
Sensory	0.035	0.55
Motor (acute)	0 182	0 18
Motor (insidious)	−0.204	0.01
Limb ataxia and/or impairment of balance	−0 287	<0 001
Diplopia and/or vertigo	0.028	0.75
C Laboratory results (controlling for age at onset, sex, motor (insidious), ataxia and/or balance, and seen at onset)		
Oligoclonal bands detected	−0.220	0.08
CSF cells > 10/mm^3	−0.051	0.72
DR2 +	−0.267	0.07
DR3 +	−0 027	0.89
Neither DR2+ or DR3+	−0.057	0.88
D Clinical course (controlling for age at onset, sex, motor (insidious), ataxia and/or balance, and seen at onset)		
Remitting at onset	0.650	<0.001
E Systems involved at last visit (controlling for age at onset, seen at onset, ataxia and/or balance, and remitting at onset)		
Visual	−0 006	0.94
Brainstem	−0.210	<0.001
Pyramidal	−0.149	0.09
Cerebellar	−0.416	<0 001
Cerebral	−0.400	<0.001
F Early clinical course (controlling for age at onset, seen at onset, remitting at onset, cerebellar, cerebral)		
No. of attacks in first 2 yrs	−0 103	<0.001
Interval between first 2 attacks	0.039	<0.001
DSS at 2 yrs	−0 415	<0.001
DSS at 5 yrs	−0.340	<0.001

($\chi^2 = 14.6$, $P < 0.001$). Progressive disease from onset was more frequent in males than females. It was also associated with slow onset of a motor deficit as an initial symptom and with spinal involvement. Advanced disability 5 yrs from onset was associated with progressive disease at onset, cerebellar involvement and whether the patient was seen from onset (data not shown).

We constructed survival curves for patients stratified according to each of the parameters independently. Most parameters that were significant in the multivariate analysis were qualitatively predictive in a similar way when studied in a univariate approach (data not shown).

Predictive models

A series of models was generated as shown in Table 2. For each model, the number of patients with missing data, the number of censored patients (did not reach DSS 6) and the number of uncensored cases whose observations generated the model are given. The log of the time to reach DSS 6 is predicted by a series of equations. For example in model 1, at diagnosis the log of the predicted median time to reach DSS 6 for a 30-yr-old male seen from onset (sex = 1; age = 30; seen from onset = 1) is: $3.92-0.175\ (1)\ -0.030\ (30)\ -0.499\ (1)\ +0.679\ (-0.367) = 2.105$. Therefore the predicted median time to DSS 6 is antilog (2.105) = 8.2 yrs. The scale result is multiplied by log (log 2) = -0.367 in order to provide the satisfactory result for the Weibull model that we have adopted. The exp (coefficient) is an indication of the effect of the parameter. For example, in model 1, male sex shortens the time to DSS 6 by exp(-0.175) or 0.84 fold. The log of the likelihood that this model correctly predicts the time to DSS 6 is shown for each model under consideration; the greater the value for the log of likelihood the better is the predictive value of the model. Note that the number of cases for which data are missing increases from model to model; this partially accounts for the increase in log of likelihood from model to model; however, the value increased even when the

TABLE 2 PREDICTIVE MODELS FOR DSS 6

Factor	*Coeff*	*SE*	*P value*
A Model 1—Demographic characteristics			
Intercept	3.92	0.101	<0.001
Male sex	−0.175	0.060	0.005
Age at onset	−0.030	0.003	<0.001
Seen at onset of MS	−0.499	0.104	<0.001
Scale	0.679	0.023	
Log of likelihood =	−898.0	Uncensored cases = 498	
		Censored cases = 562	
		Missing cases = 38	
B Model 2—Initial symptoms			
Intercept	3.91	0.102	<0.001
Male sex	−0.164	0.601	0.006
Age at onset	−0.028	0.003	<0.001
Seen at onset of MS	−0.511	0.104	<0.001
Motor (insidious)	−0.212	0.077	0.006
Limb ataxia and balance	−0.293	0.081	<0.001
Scale	0.650	0.022	
Log of likelihood =	−888.7	Uncensored cases = 498	
		Censored cases = 562	
		Missing cases = 38	
C Model 3—Systems involved			
Intercept	4.25	0.132	<0.001
Age at onset	−0.030	0.003	<0.001
Seen at onset	−0.568	0.104	<0.001
Motor (insidious)	−0.224	0.077	0.004
Brainstem	−0.184	0.061	0.003
Cerebellar	−0.430	0.073	<0.001
Cerebral	−0.255	0.100	0.01
Pyramidal	−0.230	0.090	0.01
Scale	0.648	0.022	

D

Log of likelihood = −861.5		Uncensored cases = 498 Censored cases = 562 Missing cases = 38	
D Model 4—Type of disease			
Intercept	3.35	0.122	<0.001
Age at onset	−0.021	0.003	<0.001
Seen at onset of MS	−0.680	0.102	<0.001
Remitting at onset	0.631	0.065	<0.001
Cerebellar	−0.361	0.070	<0.001
Cerebral	−0.240	0.096	0.01
Brainstem	−0.150	0.060	0.01
Scale	0.633	0.021	
Log of likelihood = −814.0		Uncensored cases = 498 Censored cases = 555 Missing cases = 45	
E Model 5—Attack interval and attacks in first 2 yrs			
Intercept	3.27	0.153	<0.001
Age at onset	−0.014	0.003	<0.001
Seen at onset of MS	−0.431	0.122	<0.001
Remitting at onset	0.449	0.067	<0.001
Cerebellar	−0.318	0.071	<0.001
Cerebral	−0.236	0.093	0.01
First interattack interval	0.026	0.006	<0.001
Number of attacks in the first 2 yrs	−0.171	0.029	<0.001
Scale	0.503	0.021	
Log of likelihood = −453.5		Uncensored cases = 307 Censored cases = 396 Missing cases = 395	
F Model 6—Attack interval and DSS at 2 yrs			
Intercept	3.55	0.116	<0.001
Age at onset	−0.009	0.003	0.001
Seen at onset of MS	−0.246	0.093	0.008
Remitting at onset	0.250	0.052	<0.001
Brainstem	−0.107	0.049	0.03
Cerebellar	−0.248	0.054	<0.001
First interattack interval	0.023	0.005	<0.001
DSS at 2 yrs	−0.381	0.016	<0.001
Scale	0.401	0.016	
Log of likelihood = −346.3		Uncensored cases = 311 Censored cases = 403 Missing cases = 384	
G Model 7—Attack interval and DSS at 5 yrs			
Intercept	3.57	0.098	<0.001
Age at onset	−0.005	0.002	0.02
Remitting at onset	0.110	0.046	0.02
Cerebellar	−0.145	0.047	0.002
First interattack interval	0.014	0.004	<0.001
DSS at 5 yrs	−0.313	0.011	<0.001
Scale	0.329	0.014	
Log of likelihood = −240.3		Uncensored cases = 282 Censored cases = 295 Missing cases = 521	

different models were fit to the same data set particularly because of the superior predictive value of DSS status at 2 and 5 yrs from onset.

Evaluation of models

To assess whether the Weibull distribution adequately accounts for random variation, a plot of log (−log (survival)) vs log time or observed minus predicted time to DSS 6 are shown for the data set as a whole (fig. 1) and after considering the covariates

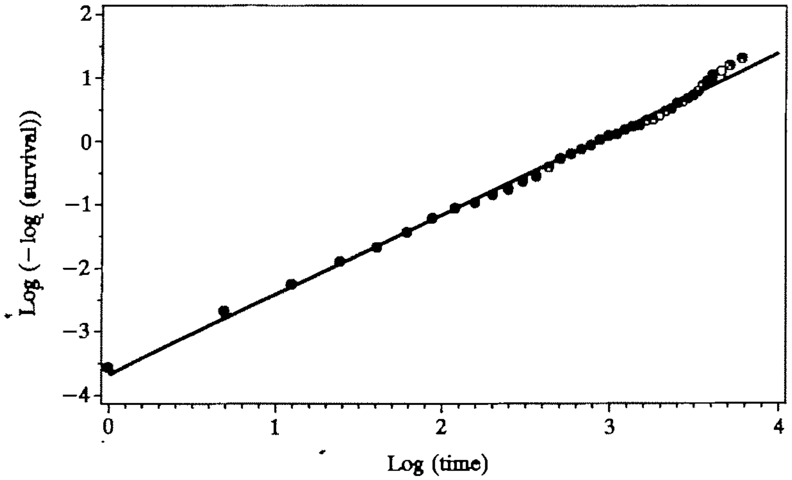

FIG. 1. Validation of the Weibull distribution Plot of observed log (−log (survival)) vs log of time (yrs), where survival is considered in terms of reaching DSS 6. No covariates are considered. Only patients having reached DSS 6 are included. Each point represents the value for patients with a unique survival estimate for DSS 6. The observations should fit the straight line for the Weibull distribution to be valid.

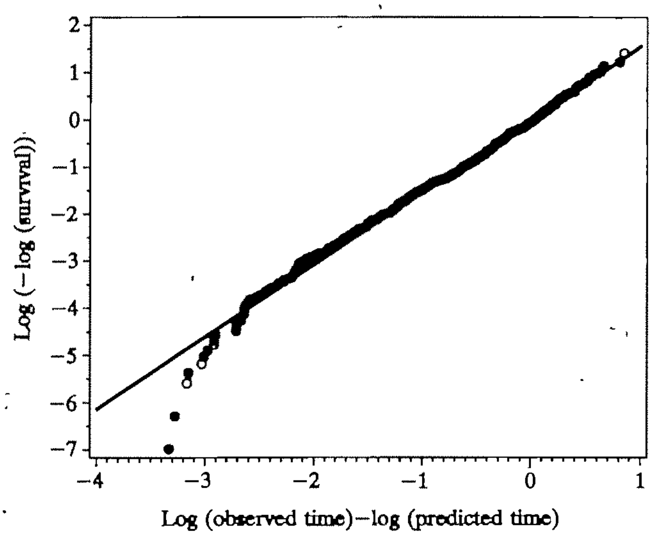

FIG. 2. Validation of the Weibull distribution for model 2 Plot of log (−log (survival)) vs log (observed time) −log (predicted time). Each point represents the value for patients with a unique set of covariates and survival estimate for DSS 6

shown in model 2 (fig. 2). In general, the Weibull distribution was quite adequate, although some patients were predicted to survive longer than they actually did, as can be seen where the observed minus predicted survival departs from the predicted linear curve in fig. 2. The distribution of errors of prediction for models 2, 5 and 6 are shown in figs 3–5. Model 2 predicted the median time to DSS 6 in 49% of cases within 6 yrs. Model 5 predicted 56% within 6 yrs and model 6 predicted 66% within that interval. We determined the extent to which these models improved the average prediction error (|predicted–observed|) compared with knowledge of the median time for the same patients (|median–observed|). The results are summarized in Table 3. Model 2 reduced the error by 12.9%, model 5 by 27.7% and model 6 by 38.8%. In this analysis, the models were all applied to the same set of patients with available data for each of the 3 models; this approach allowed comparison between models.

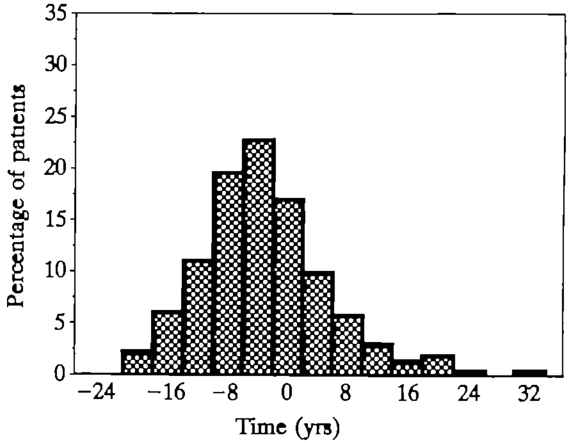

FIG 3. Distribution of prediction errors for model 2.

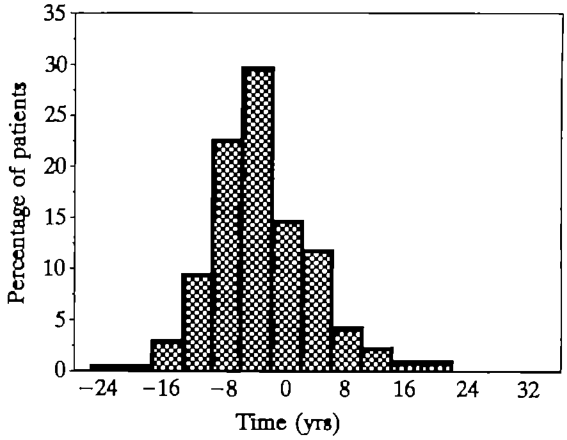

FIG. 4. Distribution of prediction errors for model 5.

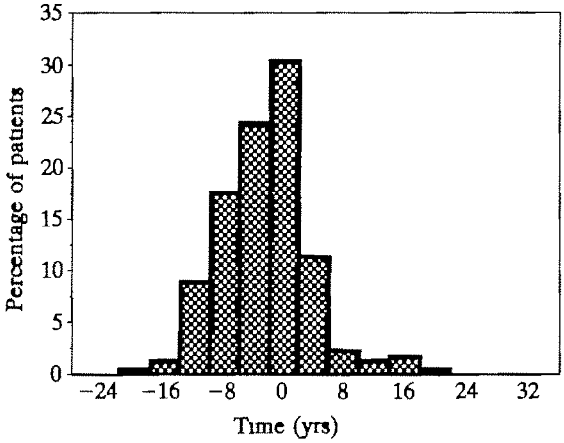

FIG 5. Distribution of prediction errors for model 6.

TABLE 3 PREDICTIVE ADVANTAGE OF MODELS FOR DSS 6

Model	*Median time to DSS 6* (yrs)*	*∣Predicted −observed∣** (yrs)*	*∣Median −observed∣** (yrs)*	*Reduction of error (%)*
2	15	7.04	8 08	12.9
5	17	6 24	8.64	27.7
6	16	5.07	8 28	38.8

* As determined for all patients with no missing values on the variables in the model
** As determined for the same set of uncensored patients (i.e , patients who reached DSS 6)

DISCUSSION

We find an association of deleterious outcome defined in terms of time to DSS 6 with several factors discernible at the onset of disease, including male sex, older age at onset, and insidious onset of a motor deficit and limb ataxia or impairment of balance as first symptoms. Optic nerve involvement, usually in the form of acute retrobulbar neuritis, is unique in that it is associated with a favourable outcome, a finding also observed by others (McAlpine, 1961; Poser *et al.*, 1982; Visscher *et al.*, 1984). Insidious onset of a motor deficit was associated with a poor outcome, likely because of an association with progressive disease from onset. Acute onset of a motor deficit, which is seen more often in cases that begin with a relapsing and remitting course, was not associated with time to DSS 6. Progressive disease from onset is highly associated with poor outcome, but even in patients with progressive disease, the DSS at 2 and 5 yrs was still highly predictive of outcome (Table 1F).

The first 2 models are based solely on data available at the patient's first assessment. The most adverse predictive parameters are being seen at onset of MS and symptoms suggestive of cerebellar disease; the former is likely to be a selection factor for patients with severe initial symptoms. Model 3 is based on observed sites of clinical deficits. The worst predictive factor in this model is the presence of cerebellar disease. Limb ataxia and difficulty with balance are no longer independent predictors of outcome because of coassociation with the presence of cerebellar disease. Similarly, male sex is no longer significant, at least in part due to the aforementioned coassociation with cerebellar disease. Model 4 incorporates information about the patient's clinical course, specifically whether remission occurred at onset of the disease or whether the course was progressive from onset. Insidious onset of a motor deficit and pyramidal tract involvement are no longer independent predictors due to coassociation with a progressive course from onset of MS. Age of onset still retains some predictive value, although the absolute value of the regression coefficient declines in successive models.

Model 5 includes the duration of the first interattack interval and the number of attacks in the first 2 yrs following onset. As the absolute value of the regression coefficients is small, the effect of the first interattack interval is negligible for the majority of patients, since the median first interattack interval by which the coefficient must be multiplied is 2 yrs (Weinshenker *et al.*, 1989*b*). For the occasional patient with a long first interattack interval, the effect on time to DSS 6 can be important. The number of attacks in the first 2 yrs (the median is 1) is a strong predictor in model 5 but is of no significance in models 6 and 7, reflecting the superior predictive value of DSS at 2 yrs and at 5 yrs. If there was little observed disability, the number of attacks in the first 2 yrs was of little consequence. The lack of independence of DSS at 2 and 5 yrs from the endpoint of DSS 6 lessens the value of these models to some degree. Obviously, a patient with fixed disability equivalent to DSS 6 at 2 yrs or greater would have a 100% chance of reaching the endpoint we considered. Nonetheless, even at 2 yrs, a DSS of 3 rather than 2 would shorten the time to DSS 6 by $\exp(-0.381) = 0.68$ fold.

We looked exclusively at time to DSS 6 for the reasons stated. Fewer than 10% of patients had reached DSS 8 at 10 yrs and fewer than 20% of patients had reached DSS 8 at 20 yrs. We cannot assume that the same results would apply if DSS 8 or DSS 10 were used as endpoint, but given the small percentage of patients who reached this level of disability, this analysis would be unlikely to yield conclusive results.

Many factors have been associated with outcome in previous studies of MS. We have reviewed these studies recently (Weinshenker and Ebers, 1987). There has been general agreement by different investigators regarding the association of a number of factors with poor outcome, including male sex (McAlpine and Compston, 1952; Panelius, 1969; Detels *et al.*, 1982), although others disagree (Leibowitz and Alter, 1970). Most previous studies find an association of poor outcome with older age at onset (Lebowitz *et al.*, 1964; Poser *et al.*, 1982; Visscher *et al.*, 1984) and with motor and cerebellar symptoms and signs at onset (Leibowitz and Alter, 1970; Kurtzke *et al.*, 1977; Visscher *et al.*, 1984). Our study corroborates these impressions to a large extent, but in addition provides the first multivariate approach designed to isolate the significance of given covariates in relation to others in a hierarchical manner. The general direction of effects of prognostic factors tested in this way is similar to the results encountered using a univariate approach, although some coassociations render various prognostic factors, such as pyramidal tract involvement, insignificant when tested with a multivariate approach. Some variables retain considerable prognostic value even in a multivariate approach including number of attacks in the first 2 yrs, first interattack interval, progressive course from onset and disability score at 2 yrs. We provide a series of predictive models which demonstrate how these data can be used to predict outcome (time to DSS 6) at either onset of MS or at various points of observation in early years of disease. The models decrease the prediction error up to 39% over knowledge of the median time. However, substantial variation in some individuals remains unexplained. Given the well-known clinical observation of substantial periods of early remission in this disease and later periods of prolonged stabilization, this observation is not surprising. As such, the utility of these models would be primarily in defining expected time to DSS 6 in groups of patients considered for clinical trials. As might have been expected, predictive accuracy of time to reach DSS 6 is refined considerably by even 2 yrs of observation. A reasonable approach in patient selection might involve selecting patients in whom the predicted time to DSS 6 is less than 5 years. The benefit of the multivariate models we have considered is that inclusion or exclusion would be based on a predicted time to DSS 6 rather than on a single clinical or demographic characteristic.

Often, however, patients are heterogeneous in disability status at entry to a clinical trial. Patients are at or near DSS 6 when considered for trials for progressive MS. The endpoint considered is usually deterioration by a single point on the DSS, assuming that deterioration by 1 point is equivalent at any point on the DSS. This assumption has not yet been studied carefully and will be explored in a final paper in this series dealing with direct applications of our natural history data in design and interpretation of results of clinical therapeutic trials.

ACKNOWLEDGEMENTS

This work was supported by a grant from the MS Society of Canada. Dr B. G. Weinshenker was a fellow of the MS Society of Canada. Drs G. C. Ebers and G. P. A. Rice are career investigators of the Ontario Ministry of Health Dr J. H. Noseworthy is a career investigator of the MS Society of Canada Rose-Anne Moore provided secretarial assistance.

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(Received January 31, 1990. Revised June 13, 1990. Accepted June 26, 1990)

Brain (11991), **114,** 1057–1067

THE NATURAL HISTORY OF MULTIPLE SCLEROSIS: A GEOGRAPHICALLY BASED STUDY

4. APPLICATIONS TO PLANNING AND INTERPRETATION OF CLINICAL THERAPEUTIC TRIALS

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SUMMARY

We used hypothetical entry criteria typical of those used in clinical therapeutic trials to determine the patients who would have been eligible among those followed in a clinic-based study of multiple sclerosis (MS) in London, Ontario, between 1972 and 1984. For these patients, we determined the observed frequency of deterioration by 1 point on the disability status scale (DSS) of Kurtzke, which is the most feasible and frequently used endpoint in clinical trials. We calculated the number of patients required for a randomized clinical trial to detect a significant result (alpha = 0.05) with 80% or 90% power based on the observed rate of deterioration. To assess the linearity of the DSS, we determined the frequency of progression and staying time at each level of the DSS. Overall the frequency of progression was lower and the staying times were longer at higher levels of disability. There was considerable intrapatient as well as interpatient variation in staying time. These data have major implications for the design and conduct of clinical therapeutic trials in MS.

INTRODUCTION

Variation in the outcome of placebo-treated or untreated groups of patients in randomized clinical therapeutic trials of immunosuppressants and other agents in multiple sclerosis (MS) complicates the interpretation of these studies. Also, the favourable outcome of placebo-treated control groups in recent studies suggests that preconceptions as to the outcome of chronically progressive MS in the short term are exaggerated, particularly in terms of the Kurtzke disability status scale (DSS) (Kurtzke, 1961) which, along with its extended version (Kurtzke, 1983), is the standard scale for assessing outcome in these trials. Several factors account for the discrepancies among different trials: variation in criteria for acceptance to clinical trials; interrater variation in assessment of disability (Noseworthy *et al.*, 1988); fluctuation in functional disability between examinations independent of permanent disability; and within and between patient variation in the rate of progression even among patients with chronically progressive disease.

We have recently described the rate of progression of disability of MS in a clinic-based population followed longitudinally at the MS Clinic, London, Ontario (Weinshenker *et al.*, 1989*a*, *b*). Furthermore, we have proposed and considered the accuracy of

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mathematical models predicting the time to reach DSS 6 in patients based on a multivariate analysis (Weinshenker *et al.*, 1991). We now discuss the practical applications of our studies of natural history to the planning and interpretation of clinical therapeutic trials in MS.

In general, patients are selected for trials of chronically progressive MS based on a single principal criterion, usually the amount of change in disability score over the previous year. There is an inherent assumption that disability will continue to progress at approximately the same rate over the ensuing years without effective medical intervention. Progression from any level to the next on the DSS is taken to be equivalent, despite the fact that it is an ordinal rather than a continuous or ratio scale.

In this paper we consider several issues which bear upon these observations. First, given a series of hypothetical entry criteria, we determined the observed rate that patients attending our clinic who satisfied these criteria reach a series of arbitrary but common endpoints used in clinical trials. Secondly, given the observed rate of progression in these patients, we calculated the number of patients that must be recruited and the required period of observation to ensure desired levels of significance and power assuming several different degrees of effectiveness of treatment. Finally, we addressed the homogeneity of the steps of the DSS by determining the duration a given patient spends at each level of disability and whether the time spent at each level of disability is at least consistent among individual patients if not between different patients.

METHODS

Data base

The clinical data base, including methods of ascertainment, has been described in previous publications (Weinshenker *et al.*, 1989*a*). Briefly, 1099 patients were followed over a mean of 11.9 ± 0.3 (mean ± SEM) yrs from onset of MS at the MS Clinic in London, Ontario, Canada. A subset of patients seen from onset who had been followed over a mean of 4.2 ± 0.2 yrs is considered separately from, as well as part of the entire data set. Evaluations of disability were longitudinal and, as such, fluctuations in disability which are problematic in short-term studies were avoided and disability scores reflected the course of disease in individual patients observed over a relatively long course of follow-up. An actuarial analysis of disability for levels DSS 3, DSS 6 and DSS 8 has been previously presented (Weinshenker *et al.*, 1989*a*).

Design of clinical trials

We considered the hypothetical entry criteria shown in Table 1. Of these criteria, A and C were more restrictive in that a deterioration of at least one point on the DSS over 1 yr was required. Criterion B was the least restrictive, requiring deterioration of 1 point over the last 2 yrs and 3 points over the last 10 yrs. Patients with disability between levels 3 and 6 were included in criteria A and B and between level 3 and 8 in criterion C. We considered 2 possible endpoints: (1) deterioration of 1 point on the DSS; (2) reaching DSS 8. All patients satisfying one set of entry criteria in a given year were selected. For each patient, a single year was randomly chosen if the patient would have been eligible in more than 1 yr. This year was then treated as the year of entry into a hypothetical clinical trial. The pooled experience of all patients who met the entry criteria was recorded for the subsequent 10 yrs. Both the entire patient population and patients seen from onset of MS were so analysed. Differences in outcome among the two groups and potential explanations for same have been previously outlined (Weinshenker *et al.*, 1989*a*). We first considered the percentage of patients that reached each endpoint. Survival analysis was used to determine the percentage that had not reached the endpoint of the study at different intervals after entry thereby accounting for right censoring of cases; we also determined the median time to the endpoint (the time at which 50% of patients reached the endpoint under consideration) (Fleiss, 1981). For a hypothetical drug that was able to increase survival (the proportion of patients not having reached the endpoint) by either 10%, 30% or 50%, the number of patients per group and length of follow-up were determined to assure results with power of 80% or 90% and a level of significance alpha = 0.05 (Donner, 1984). Similarly, the number of patients and length of study to detect an increase of 25%, 50% or 75% in median time to reach a given endpoint was determined (Donner, 1984).

Characteristics of the DSS

The distribution of patients according to disability at the time of last evaluation in the accrual of data was shown previously (Weinshenker *et al.*, 1989*a*). The distribution was bimodal with peaks at DSS 1 and DSS 6. We first determined the percentage of patients that progressed from each level of the DSS to the subsequent level. We subsequently considered the length of time patients spent at each level as they progressed through the steps of the DSS. For all patients who reached a given level of the DSS and progressed to the next, the mean time at the level was determined as well as the mean time per level of DSS. Given the documented interrater variation in terms of the DSS (Noseworthy *et al.*, 1988), we also determined the same information for the intervals DSS 1–3, 4–6, 7–8, 9–10. Finally, to address the variability within a given individual of time spent at each level of the DSS, we calculated the intraclass correlation (Zar, 1984). This is a measure of the proportion of the total variation due to variation between patients. The closer the value is to 1, the higher the correlation between staying times at different levels of the DSS for individual patients. This was first considered by ignoring the variation due to level of the DSS by including it as part of the within-patient variation. The analysis was repeated by removing this variation from the within-patient variation. Clinical correlates of variability in the rate of progression were assessed by examining the correlation of the SD of the staying time with various clinical and demographic characteristics as previously considered (Weinshenker *et al.*, 1991).

RESULTS

Design of clinical trials

The number of patients meeting the entry criteria at some point in the observed course of their disease was approximately the same for the 3 criteria considered, there being a 5% difference between the most (A) and least restrictive (B), as shown in Table 1. Most patients satisfied each inclusion criterion at some point in their illness; at any point in time, however, fewer would have qualified for A than for B. The crude percentage of patients who progressed by 1 point in terms of the DSS is shown in Table 2 for the total population and in Table 3 for the seen from onset subgroup. The use of the second endpoint, reaching DSS 8, was found to be a poor endpoint for use in clinical trials. Even when chosen according to the most stringent entry criterion A, only 17% of those selected from the total population reached DSS 8 at 10 yrs of follow-up. The results were similar for patients selected from the seen from onset subgroup where only 10% of 20 patients followed 3 yrs from hypothetical entry reached DSS 8 (data not shown). We derived a survival distribution for groups satisfying each of the 3 entry criteria.

TABLE 1 HYPOTHETICAL ENTRY CRITERIA CONSIDERED

	A	*B*	*C*
Current DSS	3–6	3–6	3–8
No. of years over which change in DSS was:			
1	1	2	1
2			3
3	5	10	
No of eligible patients	635	668	657

TABLE 2. OBSERVED PERCENTAGE OF PATIENTS IN TOTAL POPULATION DETERIORATING BY 1 LEVEL ON THE DSS BASED ON ENTRY CRITERIA

Years after entry	*Percentage of patients (n)*		
	Criterion A	*Criterion B*	*Criterion C*
0	0 (635)	0 (668)	0 (657)
1	30 (558)	35 (586)	32 (574)
2	46 (465)	48 (474)	43 (468)
3	54 (381)	57 (390)	52 (390)
4	62 (329)	64 (324)	58 (324)
5	70 (284)	69 (271)	67 (276)
6	76 (241)	72 (226)	70 (226)
7	76 (199)	72 (190)	72 (185)
8	79 (165)	74 (154)	74 (142)
9	81 (126)	77 (125)	76 (118)
10	85 (112)	84 (110)	82 (104)

n = number of patients with follow-up data.

TABLE 3. OBSERVED PERCENTAGE OF PATIENTS IN SEEN FROM ONSET SUBGROUP DETERIORATING BY 1 LEVEL ON THE DSS BASED ON ENTRY CRITERIA

Years after entry	*Percentage of patients (n)*		
	Criterion A	*Criterion B*	*Criterion C*
0	0 (60)	0 (60)	0 (59)
1	51 (47)	51 (47)	44 (48)
2	72 (29)	81 (16)	58 (24)
3	84 (19)	80 (10)	64 (14)
4	73 (11)	88 (8)	50 (8)
5	75 (8)	100 (4)	57 (7)
6	83 (6)	100 (1)	75 (4)
7	100 (4)	100 (1)	100 (3)
8	100 (2)	– (0)	– (0)

n = number of patients with follow-up data.

The percentage that did not deteriorate by a further 1 point on the DSS ('survival') is shown in Table 4, along with the median time at which 50% of those who entered reached that endpoint.

Based on values representative of those in Table 4, the number of patients required in each of two randomly assigned groups in a controlled study of a therapeutic agent designed to evaluate efficacy with either 80% or 90% power at a level of significance alpha = 0.05 is shown in Table 5. Similarly, we determined the number of patients necessary to detect an increase in median time to deteriorate by 1 level on the DSS by 25%, 50% and 75% (Table 6). The same degrees of power and significance were considered. The data are shown for differing recruitment periods.

We analysed the data to determine differences in the clinical and demographic characteristics of those who satisfied and those who did not satisfy the entry criteria. The characteristics of those who satisfied the entry criteria were generally the same as those which predicted a poor outcome in our previous study of prognostic indicators

TABLE 4 SURVIVAL ANALYSIS PERCENTAGE OF PATIENTS WHO HAVE NOT DETERIORATED BY 1 LEVEL ON THE DSS FROM TIME OF ENTRY

	Percentage of patients		
Years after entry	*Criterion A*	*Criterion B*	*Criterion C*
Total population			
1	71	67	70
2	54	53	57
3	43	42	45
4	35	33	37
5	27	28	29
6	21	22	23
7	17	20	20
8	14	16	17
9	11	14	15
Median time (yrs)	2.3	2.2	2.6
Seen from onset			
1	55	55	61
2	37	26	41
3	26	26	32
4	26	17	32
5	26	9	24
Median time (yrs)	1.3	1.2	1.6

TABLE 5 NO. OF PATIENTS PER GROUP TO DETECT AN INCREASE OF 0.10, 0.30 OR 0.50 IN THE PROPORTION OF PATIENTS WHO REMAIN STABLE

		*No. of patients**		
Years after entry	*Control rate*	*0.10*	*0.30*	*0.50*
Total population				
1	0.70	332 (431)	–	–
2	0.55	415 (542)	48 (60)	–
3	0.45	430 (563)	53 (67)	19 (23)
4	0.35	415 (542)	55 (69)	21 (25)
5	0.30	395 (515)	54 (68)	22 (26)
7	0.20	332 (431)	51 (64)	22 (26)
9	0.15	289 (373)	48 (60)	21 (25)
Seen from onset				
1	0.60	395 (515)	44 (54)	–
2	0.40	426 (557)	54 (68)	20 (24)
3	0.30	395 (515)	54 (68)	22 (26)
4	0.25	367 (478)	53 (67)	22 (26)
5	0.20	332 (431)	51 (64)	22 (26)

* To assure level of significance alpha = 0.05 and power 80% (90%).

(Weinshenker *et al.*, 1991). In comparison with those who did not, those who qualified for entry criterion A were older (32.4 ± 0.4 vs 27.75 ± 0.4 yrs), more frequently presented with insidious onset of a motor deficit (20 ± 2% vs 6 ± 1%) and at a higher mean DSS score at 5 yrs from onset (3.11 ± 0.09 vs 1.54 ± 0.08).

TABLE 6 NO. OF PATIENTS PER GROUP TO DETECT AN INCREASE OF 25%, 50% AND 75% IN MEDIAN TIMES TO PROGRESSION OF 1 LEVEL ON THE DSS

Duration of study (yrs)	*Recruitment period*	*Control time (yrs)*	*No. of patients**		
			25%	*50%*	*75%*
Total population					
2	1	2.4	741 (993)	244 (327)	138 (185)
3	1		523 (701)	171 (229)	97 (130)
	2		613 (821)	201 (269)	114 (152)
5	1		391 (523)	127 (170)	71 (95)
	2		412 (552)	134 (179)	75 (101)
	3		443 (594)	144 (193)	81 (109)
	4		493 (660)	161 (215)	91 (121)
Seen from onset					
2	1	1.2	475 (637)	155 (208)	87 (117)
3	1		377 (506)	122 (163)	69 (92)
	2		421 (564)	136 (183)	77 (103)
5	1		331 (443)	106 (142)	59 (79)
	2		337 (452)	108 (145)	61 (81)
	3		350 (468)	112 (151)	63 (84)
	4		374 (501)	121 (162)	68 (91)

* To assure level of significance alpha = 0.05 and power 80% (90%)

TABLE 7 NO OF YEARS AT EACH LEVEL OF DSS FOR THE PATIENTS WHO REACH THE NEXT LEVEL

DSS	*Number entering*	*Number progressing*		*Time at DSS (yrs)*			*Mean time per level of DSS*		
		n	*%*	*Mean*	*SEM*	*Median*	*Mean*	*SEM*	*Median*
Total population									
1	1037	854	82	4.09	0.19	1	2.78	0.08	2.17
2	829	675	81	2.80	0.14	1.5	2.44	0.07	2.00
3	662	539	82	1.95	0.11	1	2.17	0.06	1.86
4	536	467	88	1.22	0.06	1	2.07	0.06	1.83
5	475	444	94	1.25	0.06	1	2.06	0.06	1.81
6	489	292	60	3.06	0.17	2	2.06	0.07	1.86
7	306	114	37	3.77	0.32	3	1.87	0.10	1.65
8	114	32	28	2.41	0.38	2	1.61	0.17	1.42
9	34	14	41	2.51	0.56	2	1.41	0.27	1.25
10	16	–	–	–	–	–	–	–	–
Seen at onset									
1	196	125	64	1.77	0.18	1.0	1.46	0.11	1.00
2	125	72	58	1.29	0.19	0.75	1.04	0.08	0.83
3	71	52	73	0.94	0.17	0.5	0.89	0.07	0.73
4	51	46	90	0.63	0.08	0.5	0.83	0.06	0.71
5	45	44	98	0.75	0.09	0.5	0.80	0.06	0.71
6	44	26	59	1.43	0.24	1.0	0.77	0.08	0.71
7	26	10	38	1.65	0.53	1.0	0.89	0.14	1.00
8	10	4	40	1.31	0.43	1.5	0.66	0.22	0.61
9	4	1	25	0.25	0	0.25	0.20	0	0.20
10	1	–	–	–	–	–	–	–	–

Characteristics of the DSS

We determined the percentage of patients who progressed to the next level of disability among those who entered each level of DSS. For these patients, we determined the mean and median times that they remained at a specific level. We also determined the mean and median times which they spent at any of the levels of disability on the DSS (Table 7). Small differences between numbers of patients progressing from given levels of the DSS and numbers of patients entering subsequent levels are due to patients with missing values for the time to certain levels of the DSS. Patients were deemed to have remained at a given level on the DSS until it was recorded that they reached a higher level. Generally, the percentage of patients progressing to a higher level of disability decreased inversely with the level of disability. The mean staying times were greatest at DSS 1 and DSS 7 and were least for DSS 4 and DSS 5. This is consistent with the cross-sectional distribution of disability that we have previously reported (Weinshenker *et al.*, 1989*a*) wherein we found that the distribution of disability was bimodal with relative peaks at DSS 1 and DSS 6. The difference between the mean and median, which is a measure of the skew in the frequency distribution according to time at each level of the DSS, was considerable for DSS 1 but small at all levels greater than DSS 4. The data suggest a large skew to the right in the frequency distribution of staying time at DSS 1. Patients who had reached DSS 3 had spent an average of 2.17 ± 0.06 yrs (mean ± SEM) at each level to that point, while patients at DSS 6 had spent 2.06 ± 0.06 yrs and patients at DSS 8 had spent 1.61 ± 0.17 yrs, reflecting the higher percentage with aggressive disease. The trend toward longer staying times at DSS 1 and DSS 7 was similar for the seen from onset subgroup. The relatively shorter durations spent at each level reflects the shorter mean period of follow-up (4.2 ± 0.2 yrs).

This analysis was repeated arbitrarily dividing the DSS into levels 1–3, 4–6, 7–8, 9–10 (Table 8). A smaller percentage of patients in the seen from onset group progressing

TABLE 8 NO OF YEARS AT EACH LEVEL OF DSS* FOR THE PATIENTS WHO REACH THE NEXT LEVEL

		Number progressing		*Time at DSS (yrs)*			*Mean time per level of DSS*		
DSS	*Number entering*	*n*	*%*	*Mean*	*SEM*	*Median*	*Mean*	*SEM*	*Median*
Total population									
1–3	1012	547	54	7.96	0.31	6	5.57	0.15	5.00
4–6	543	271	50	5.10	0.22	4	4.74	0.17	4.33
7–8	308	33	11	5.04	0.62	4	3.86	0.39	3.75
9–10	34	–	–	–	–	–	–	–	–
Seen at onset									
1–3	195	52	27	2.85	0.31	2.17	2.29	0.18	1.88
4–6	52	26	50	2.73	0.32	2.40	1.87	0.20	1.67
7–8	26	4	15	2.19	0.80	2.13	1.50	0.49	1.38
9–10	4	–	–	–	–	–	–	–	–

* Modified DSS scale.

beyond DSS 3 reflects the higher percentage that remain purely relapsing remitting over the relatively short period of follow-up.

Finally, we considered the within and between-patient variation of the staying time at each level of the DSS. This was expressed by the intraclass correlation. Only patients with staying times defined for 67% of the levels of the DSS less than the final level at the time of analysis were considered. We chose to consider only patients between DSS 3 and 6 and DSS 6 and 8, as patients at lower levels of disability (DSS 1–3) are generally in a relapsing and remitting phase of illness and generally have a large intraindividual variability in staying time. This was evident in the difference between the mean and median staying time shown in Table 7. The intraclass correlations are presented in Table 9. Significant intraclass correlation was not demonstrated except for DSS 6–8 in the seen from onset subgroup; data could only be analysed in 4 patients in this group, however. This shows that the within-patient variability in staying time at different levels of the DSS is substantial.

TABLE 9 INTRACLASS CORRELATION ANALYSIS

	n	*A**	*B**
Total population			
DSS 3–6	258	0.17	0.10
DSS 6–8	30	0.14	0.14
Seen at onset			
DSS 3–6	26	0.10	0.08
DSS 6–8	4	0.77	0.64

* Variation in staying times at different levels of DSS was considered (A) or not considered (B) as part of within-patient variation

We performed a correlation analysis of the SD of the staying time with clinical and demographic patient characteristics to identify those associated with intrapatient variation in staying times (data not shown). Patients with longer intervals between initial attacks, fewer attacks in the first 2 yrs and lower levels of disability at 2 and 5 yrs from onset of MS tended to have more variable progression in terms of SD of staying time. Patients with younger age at onset and those with relapsing and remitting disease from onset also had a more variable course with respect to staying times.

DISCUSSION

While other criteria have recently been proposed for monitoring outcome of clinical trials, including MRI (Paty, 1988) and quantitative evoked potentials (Nuwer *et al.*, 1987), the standard for success must remain clinical response. The standard for measuring the clinical response in MS is the DSS, or more recently the expanded (EDSS) (Kurtzke, 1983). Numerous randomized clinical trials have failed to show benefit in terms of the DSS in part because the control untreated group had done unexpectedly well (British and Dutch Multiple Sclerosis Azathioprine Trial Group, 1988; Ellison *et al.*, 1989; Miller *et al.*, 1989), at least in the short term. Other studies have failed to show differences between an experimental therapy and 'standard therapy'. Kappos *et al.* (1988) failed to show differences between cyclosporin A and azathioprine in patients with progressive MS. One can only speculate whether either agent altered the natural history of MS.

Our approach in generating patient cohorts was artificial, in that patients who qualified in any point in their course according to the entry criteria were assumed to have done so simultaneously and were entered into a single hypothetical trial. As the size of the cohorts was greater than 600 and differed little whether selected according to criteria A, B or C, it may be inferred that most patients would qualify for each hypothetical trial at some point. At any point in time, however, fewer would qualify for criterion A than for B. Our results suggest that a clinical trial involving 120 patients selected from our total population, randomized to two groups, should demonstrate a significant difference 90% of the time in terms of the DSS at the end of 2 yrs assuming that the experimental drug could decrease the percentage progressing by 1 point by 30%. Similarly, a drug which could decrease the rate of progression by 50% would result in a significant difference within 3 yrs if 46 patients were randomly assigned. More stringent endpoints such as reaching DSS 8 occur too infrequently and require unrealistically long follow-up periods. Lesser periods of observation would not be useful as the control rate of deterioration is too low at an earlier point to detect a difference reliably.

The observed deterioration of 30% of cases by 1 point on the DSS at 1 yr and 54% at 3 yrs in patients selected according to criterion A is somewhat worse than that seen in control placebo limb of a recent azathioprine-methylprednisolone trial where 41% (9/22) deteriorated by 1 EDSS point at 3 yrs follow-up (Ellison *et al.*, 1989). In a recent copolymer-1 trial (Miller *et al.*, 1989) in patients with progressive MS, 29.5% of patients in a placebo limb deteriorated by 1 EDSS point at 2 yrs compared with 46% in our data. Other trials have produced quite different results in their control groups, even when not placebo treated. Hauser *et al.* (1983) found that 80% of ACTH-treated patients with chronically progressive MS deteriorated by 1 point on the EDSS or by 1 point on an ambulation index at 1 yr of follow-up.

Our data may not entirely apply to patients who participate in clinical trials because of the placebo effect of participation and because of selection factors such as compliance which generally are associated with a favourable outcome. The phenomenon of 'regression to the mean' may apply to patients selected for a clinical trial based on rapid deterioration in the year of selection. Despite deterioration of 1 or more points over the previous year, chance favours return to the baseline of deterioration by 0.5 DSS/yr, which is the mean rate of change in MS patients in the first 5 yrs after a progressive course intervenes (Weinshenker *et al.*, 1989*a*). This is a further explanation for the unexpectedly favourable course of patients with progressive MS in the placebo limbs of clinical trials. Our data would support this concept. The lack of difference in outcome (Tables 2–4), regardless of the stringency of the entry criteria, suggests that criteria based on progression of disability over the previous 1–5 yrs are of limited usefulness in identifying patients likely to continue to progress rapidly.

Many undefined factors may generate differences between groups selected for similar degrees of measurable deterioration over 6–12 months prior to entry. First, variability of progression among different individuals, variation from examination to examination for a given individual, and interrater variability may contribute. Demonstration of deterioration in terms of the DSS on more than one occasion may mitigate the problems with reproducibility. In terms of this study, as disability was determined longitudinally, and times to reach successive levels on the DSS were recorded, our data were relatively free of fluctuation in scores unrelated to permanent disability. Secondly, differences in baseline disability may also contribute, particularly with regard to differences in the rate of progression from and staying times at different levels of the DSS. We addressed the latter aspect in this analysis.

The frequency of progression was lower and the staying times were longer at higher levels of disability on the DSS than at lower levels. This is the expected correlate of the cross-sectional distribution of disability of our clinic population that we have described previously. The frequency distribution was bimodal with relative peaks at DSS 1 and DSS 6. The peaks of cross-sectional disability occur at levels with the longest staying time and/or the lowest frequency of progression to subsequent levels. Progression not only varies among different individuals, but also within a given individual from level to level as evidenced by the low intraclass correlation. The implication for clinical trials is that the progression from DSS 5 to DSS 6 carries different significance than progression from DSS 6 to DSS 7. This must be accounted for by stratification at entry. Furthermore, change in the mean EDSS which has been used as an important if not the principal endpoint in many studies is methodologically incorrect (Kurtzke, 1986). Differences in the proportion of patients changing by a given degree of disability and in the duration over which this occurs are more methodologically correct. Our data suggest that, in addition, the data may have to be stratified for level of baseline disability.

How then should patients be selected for clinical trials? No single factor appears to discriminate patients who will pursue a benign or aggressive course. Nonetheless, a combination of factors such as illustrated in our previous multivariate analysis (Weinshenker *et al.*, 1990) provides a rational basis for selection of patients at an earlier point in their disease, perhaps at DSS 3. At this point, the staying time is shorter, yet the variability in staying time is less than that seen in the very early stages of MS (DSS 1 and DSS 2). However, in clinical trials conducted over a relatively short term of 1–2 yrs with patients at different levels of disability at entry, deterioration of 1 point on the EDSS is the most feasible endpoint. In these circumstances, differences in baseline disability must be considered in analysis.

The natural history of MS must be the standard by which the result of any clinical trial is assessed. The natural history of any disease can only be understood in terms of the tools with which this disease is measured. The DSS and EDSS are the principal tools used at this time until replaced by other validated and improved biological measures of disease burden. Our data provide a basis for rational interpretation and design of clinical therapeutic trials in MS.

ACKNOWLEDGEMENTS

Dr Brian G. Weinshenker was a fellow of the MS Society of Canada. Drs George Ebers and George Rice are career investigators of the Ontario Ministry of Health. Dr John Noseworthy is a career investigator of the MS Society of Canada. This work was supported by a grant from the MS Society of Canada. Louise Laniel provided expert technical assistance in the preparation of the manuscript. Dr D. Paty played an important role in the early phase of these studies (I–IV).

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(*Received December 19, 1989. Revised June 13, 1990. Accepted June 26, 1990*)

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MAGNETIC RESONANCE IMAGING OF EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS IN PRIMATES

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SUMMARY

The potential use of quantitative MRI to characterize early, as well as late, immune-mediated lesions of multiple sclerosis (MS) has been investigated. Experimental allergic encephalomyelitis (EAE) was induced in 4 male Macaca fascicularis monkeys, and the development of the disease followed using quantitative MRI at 0.15 Tesla. Serial scans were recorded daily, beginning at day 9 after inoculation. Lesions were detected before the onset of clinical signs, due to an elevation in the spin-lattice (T_1) and spin-spin (T_2) relaxation times. The T_1 and T_2 values from the lesions were shown to increase over time, indicative of progressive change at a molecular level. The appearance of any new lesions and any changes in existing ones were noted; this allowed dating of the lesions postmortem. Pathological correlation showed the long T_1 and T_2 values to be associated with the presence of inflammation, demyelination and haemorrhagic necrosis. Microscopically similar lesions had the same MRI characteristics. In addition, these studies showed the oldest lesions to be the most haemorrhagic; this is contrary to the belief that haemorrhage is a secondary event in EAE. The results show that quantitative MRI is sensitive to variable pathology, and has the potential for use in characterizing the pathological progression in MS.

INTRODUCTION

Multiple sclerosis (MS) is characterized by demyelination in the central nervous system (CNS) (Matthews *et al.*, 1985). Pathological and clinical studies are numerous (Prineas and Connell, 1978; Confavreux *et al.*, 1980; Prineas, 1985; Kurtzke and Hyllested, 1986), yet little is known about the pathological progression of the disease. This problem exists because the only pathological data available, except for a few biopsies, are postmortem, by which time the disease is usually in the chronic stage.

Experimental allergic encephalomyelitis (EAE) is an induced animal disease in which attempts have been made to understand the mechanisms of development of early, as well as late, immunologically-mediated, inflammatory and demyelinating lesions in the CNS. EAE can be produced in susceptible species and strains by sensitization to white matter or to myelin basic protein (MBP) in Freund's complete adjuvant (FCA) (Raine, 1984). MBP makes up 30–35% of the proteins in normal CNS myelin. Manipulation of the experimental conditions for induction of EAE allows the production of not only

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hyperacute and acute forms but also chronic and remitting-relapsing courses of the disease (Alvord, 1970, 1985) which may represent an animal model for MS.

In man, MS can run several different clinical courses: acute, chronic progressive, chronic relapsing-remitting, or various combinations thereof. Histologically, the disease is characterized by relatively sharply defined demyelinative lesions with preservation of axons. EAE can also present with a variety of clinical courses. The classical form is usually an acute disorder with inflammatory and/or demyelinating-necrotic processes depending on the species studied: in guinea pigs and rats it is almost purely inflammatory, whereas in rabbits and monkeys it is both inflammatory and demyelinating, and in monkeys even necrotic. The early lesions are relatively small and perivascular but they may coalesce into large irregularly shaped ones. Manipulation of the experimental protocol allows the production of hyperacute and chronic forms of the disease.

Monkeys sensitized with MBP in FCA may show all three forms: hyperacute, acute or chronic, the last being the least common unless treatment is given to prolong the course. Thus most monkeys sensitized to MBP in FCA die either with hyperacute or acute EAE. Hyperacute EAE in monkeys is marked by severe inflammation (with neutrophils, haemorrhage, oedema and fibrin), demyelination and varying degrees of necrosis. Although many of these changes resemble acute necrotizing haemorrhagic leucoencephalopathy in humans, some lesions resemble acute MS. We have studied primate EAE in order to observe the evolution of acute immunologically-mediated CNS lesions visible on magnetic resonance imaging (MRI). In addition, chronic EAE in monkeys has many more pathological similarities with MS and should provide us with more insight concerning the MRI correlations with MS.

In order to make full use of the knowledge gained from studying EAE there must be some way of correlating the features of EAE and MS in vivo. MRI can help towards the achievement of that goal. MRI has already been shown to be a powerful tool in the study of MS in vivo (Young *et al.*, 1981; Cutler *et al.*, 1986; Farlow *et al.*, 1986; Haughton *et al.*, 1986; Ormerod *et al.*, 1987; Paty *et al.*, 1988), and NMR spectroscopy has been used in the study of experimental models (Karlik *et al.*, 1986; Richards *et al.*, 1988). In the present study, we have used both qualitative and quantitative MRI to study primate EAE with a goal of learning how to interpret the MRI information from the acute inflammatory and demyelinating CNS lesion of MS.

METHODS

Induction of EAE

Permission for carrying out these studies was granted by the Animal Care Committee at the University of British Columbia. Four male Macaca fascicularis monkeys, weighing 3–5 kg, were fed a regular diet of monkey chow and fresh fruit before and after induction of EAE. EAE was induced by intradermal injection of 0.15 ml of a water-in-oil emulsion containing 0.5 mg of Mycobacterium tuberculosis and 15 mg of MBP, divided among 3 sites in the hind foot pads (Alvord *et al.*, 1979). The MBP was extracted using the batch method of Deibler *et al.* (1984). Clinical observations of the monkeys were made 3 times daily beginning 9 days after inoculation (Table 1) (Alvord *et al.*, 1984).

MRI protocol

MRI data were collected on a Picker International NMR Cryogenic '2000' system, operating at a field strength of 0.15 Tesla (proton resonance frequency 6.4 MHz); a receiver coil (saddle design, built by Picker International) with an aperture of 15 cm was used to obtain the images. Multislice spin-echo (SE)

TABLE 1. CLINICAL SCORING FOR THE DEVELOPMENT OF EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS IN MONKEYS

1. ?: Prodromal signs

Anorexia (food pellets scattered on the floor of the cage)
Weight loss
Inactivity
Slow response to startle
Irritability
Yawning

2. ±: Mild neurological signs

Drooling
Nystagmus
Transient tremor
Clumsiness
Head tilt
Apathy (indifference)
'Headache' (acute stress)

3. +: Moderate neurological signs

Pupillary abnormalities (unequal, dilated, poorly reactive to light)
Ptosis
Strabismus
Tremor
Seizure
Paresis
Incontinence
Body twisting
Blindness
Ataxia
Akinesia

4. + +: Severe neurological signs

Paraplegia
Hemiplegia
Quadriplegia
Lethargy or somnolence

5. + +: Moribund

Semicoma
Coma
Decerebration
Decortication

6. + + + +: Death

and inversion-recovery (IR) pulse sequences were employed, both of which provided 8 contiguous, 5 mm thick slices. Echo-delay times (TE) of either 40 and 60 ms, or 40 and 80 ms were used in the SE sequences. In the IR sequences, the 180° – 90° interpulse delay (TI) was 400 ms. In all sequences the repeat time (TR) was 2 s. Spin-echo sequences are referred to as $SE_{TR/TE}$ in all figures.

The monkeys were positioned in the scanner using the laser available for patient alignment, such that the inner canthi of the eyes were aligned perpendicular to the direction of the static magnetic field; the appropriate flexion of the neck was determined visually. For comparisons to be made, repositioning was critical in the serial scans; a sagittal pilot scan was obtained in 0.8 min, allowing the position of the monkey to be checked and positioning altered if necessary. The pilot scan also allowed the centre of the first transverse slice to be positioned on the superior surface of the brain; this was important for subsequent comparison with the gross and microscopic lesions seen pathologically.

The pulse sequences were chosen to allow direct comparison with human MS data, and also to allow the computation of the spin-lattice relaxation time (T_1) and the spin-spin relaxation time (T_2) (Farrar and Becker, 1971); the latter computations were performed using the standard Picker International software (Condon *et al.*, 1986). The quantitative MRI data were transferred from magnetic tape to a VAX-11/750 computer, the bus of which connects to a Ramtek 9460 multiuser image display system, which has a black-and-white display channel with a resolution of 640 × 512 pixels. Peripherals are available for program control functions such as menu selection, and for data entry functions such as contour tracing and feature measurement from the display screen. Regions of interest (ROI) were outlined using a trackball cursor controller. T_1 and T_2 values were obtained from the computed images by outlining the area of 15 pixels at the centre of the lesions or from an area of normal appearing white matter. The same lesions were followed serially and, where possible, lesions that extended through 3 slices were chosen to minimize the effects of volume-averaging.

The monkeys were anaesthetized for scanning using a 12:1 combination of ketamine hydrochloride (Roger/STB Inc.; chemical name ±-2-o-chlorophenyl-2-methylaminocyclohexanone hydrochloride, 100 mg/ml) and rompun (Haver-Lockhart; chemical name 2-(2,6-dimethylphenylamino)-4H-5,6-dihydro-1,3-thiazine, 20 mg/ml) (0.3 mg/kg). Since the monkeys were supine while being scanned, atropine (Clark Rogers Ltd, 0.5 mg/ml) was also administered at the time of anaesthesia (0.2–0.4 ml) to prevent oral fluid accumulation and aspiration.

The monkeys were scanned before inoculation and then daily from day 9 after inoculation. Before each set of scans were obtained, the monkeys were checked for evidence of neurological impairment. Following the earliest detection of changes on MRI (as indicated by increased signal intensity on the SE images; Young *et al.*, 1983), images were obtained 3 times daily until death. T_1 and T_2 computations were carried out for each set of MR images and the values recorded for the abnormal areas. The ranges of T_1 and T_2 corresponding to normal-appearing white matter were obtained from statistical analyses on 8–24 areas from the equivalent slice in 3 serial scans; both are comparable to those seen in humans (Kjos *et al.*, 1985). When each monkey became incapacitated, unable to feed or drink by itself, it was killed using 3 ml of intracardiac euthanyl (MTC Pharmaceuticals) while under anaesthetic. When possible, immediate premortem and postmortem scans were obtained. The brain was then removed and fixed in 10% neutral buffered formalin. After a period of at least 1 wk a set of MR images was obtained from the fixed brain. The brain was positioned on its superior surface and a sagittal pilot used to check its positioning. The centre of the first horizontal slice was positioned on the superior surface of the brain, thereby allowing direct comparison between the images of the fixed brain with both the in vivo images and the gross and microscopic pathology.

Pathology

Using the sagittal pilot scan to orient the brain visually, horizontal brain slices, each 5 mm thick, were cut using a standard brain knife guided by 5 mm outside diameter glass rods; the inferior surface of each slice corresponded to the centre of the equivalent MRI. After comparison of the gross lesions with the MRI data, appropriate blocks were taken for microscopic evaluation, embedded in paraffin and stained with haematoxylin and eosin (H and E), Luxol fast blue combined with periodic acid-Schiff and haematoxylin (LFB-PAS-H), with Holmes' axon stain (LFB-Holmes) or with Nissl's stain (LFB-Nissl) and gallocyanin for myelin combined with Darrow red (gallo-DR) In addition, frozen sections were stained with oil red O and haematoxylin (ORO-H).

RESULTS

Case 1

MRI and clinical observations. An area of increased signal intensity on the SE images (TE = 40 ms and 80 ms) was observed in the brainstem (midbrain) 15 days after inoculation. This change was associated with the onset of lethargy and bilateral ptosis. Over the course of the next few days there was little change in the MRI (fig. 1) and the monkey remained clinically stable; hence the monkey was only scanned once a day on days 17–21. On day 21 after inoculation, the MRI lesion spread rostrally and caudally. This change was associated with worsening of the ptosis in the left eye.

On day 22, the MRI remained positive in the same 3 slices and the ptosis in the left eye was worse. By late evening of the same day, the monkey was off balance and the right arm appeared spastic.

On day 23 the monkey lay prostrate in the cage. He was nursed all day to see if he would recover but had to be killed at 7.35 p.m. An immediate postmortem set of scans was obtained. The brain was removed and immediately placed in 10% neutral buffered formalin. The lesion, previously easily seen on the MRI (both SE and IR), could not be seen in the images of the fixed brain. This is in contrast to our experience with brains from humans with chronic MS (Stewart *et al.*, 1984, 1986), in which the demyelinated and gliotic lesions were easily and reproducibly seen on both immediate postmortem and fixed brain scans.

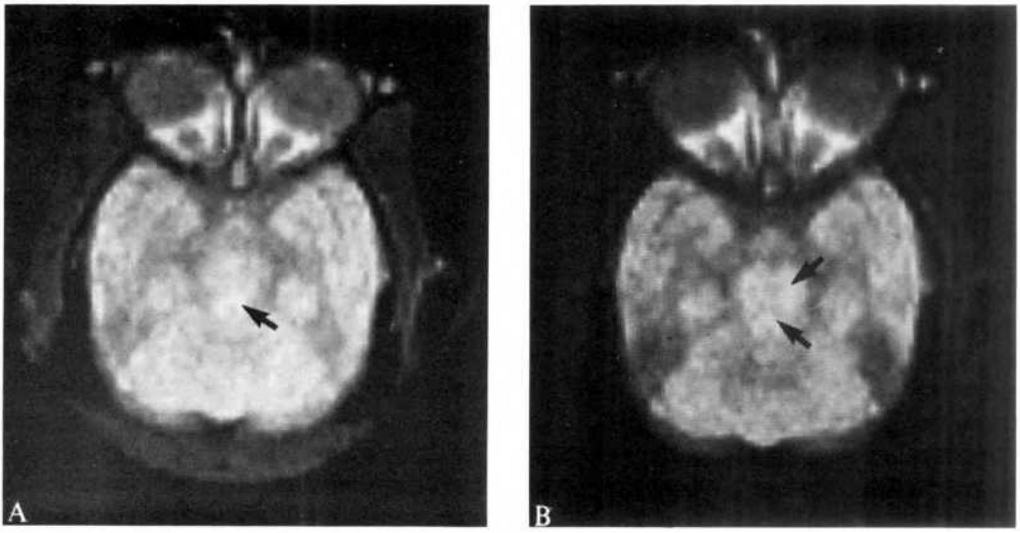


FIG. 1. Serial $SE_{2000/40}$ MRI obtained from *Case 1*. A, day 15 after inoculation. The arrow shows the lesion in the brainstem. B, day 18 after inoculation. The arrows show the spread of the lesion in the brainstem.

Comparison of the MRI with the gross distribution of lesions. Photographs of the 5 mm thick brain sections and the histological preparations were compared with the equivalent MR images, as shown in fig. 2. A 10×10 mm lesion, mainly in the midbrain and extending into the rostral pons, could be seen in the gross sections. As was observed on the MRI, the lesion was present in three 5 mm thick brain sections. Another small lesion consisting of inflammation surrounding about 12 blood vessels over an area of 5×2 mm could be seen in the left occipital subcortical white matter; this small lesion was not detected on the MRI.

Histology. The initial area of increased signal observed on the SE image was found to correspond to a confluent perivascular inflammatory lesion, in which the vessel walls were infiltrated by white blood cells diffusing out into the tissue (fig. 3A). Most of the cells were polymorphonuclear leucocytes with a small number of lymphocytes and a large number of macrophages; most of the macrophages were large round cells (gitter cells) filled with granular sudanophilic lipids (fig. 4) and LFB-positive myelin debris (fig. 3B). There was no haemorrhage in this lesion but there was evidence of subtotal necrosis with swelling, beading and destruction of axons (fig. 3D). At the edges of the lesion, the myelin sheaths were remarkably swollen (fig. 3C).

Quantitative MRI. The T_1 and T_2 values from the lesion were measured as a function of time after inoculation (fig. 5) and were shown initially to increase, then stabilize while the monkey was clinically stable.

Case 2

MRI and clinical observations. An area of increased signal on the SE images (TE = 40 ms and 80 ms) was observed in the left cerebral hemisphere on day 22 after

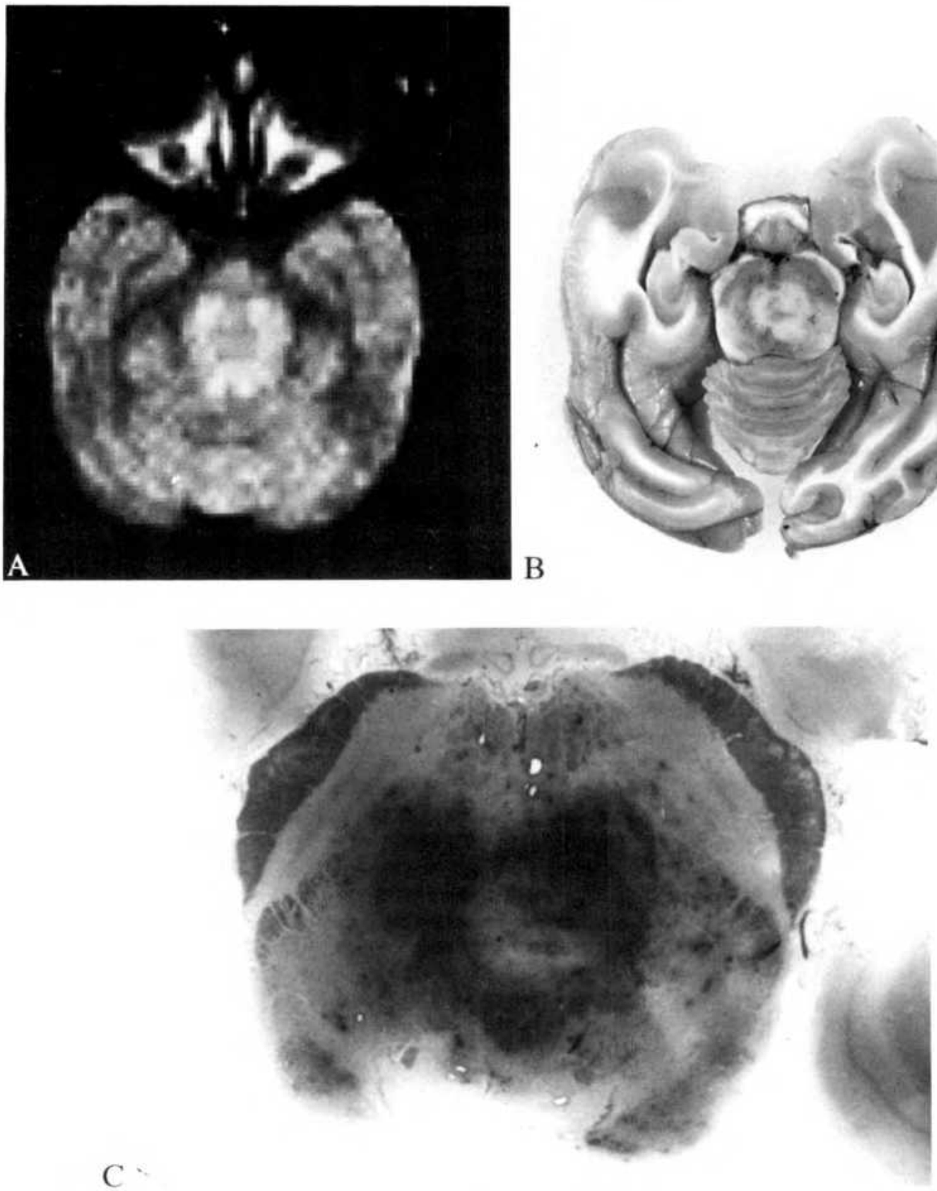


FIG. 2. Comparison of MRI with the gross pathology in *Case 1*. A, SE$_{2000/40}$ MRI showing lesion in the brainstem. B, inferior surface of 5 mm thick brain slice at the same level. C, ORO-H (×4) preparation at the same level.

inoculation (fig. 6A). A noncontrast CT scan obtained the same day did not show evidence of haemorrhage or low-density. The monkey was killed on that day and an immediate postmortem scan obtained. The appearance of this abnormal area was not associated with any neurological signs apart from a small loss in weight. The lesion was not visible on the images of the fixed brain.

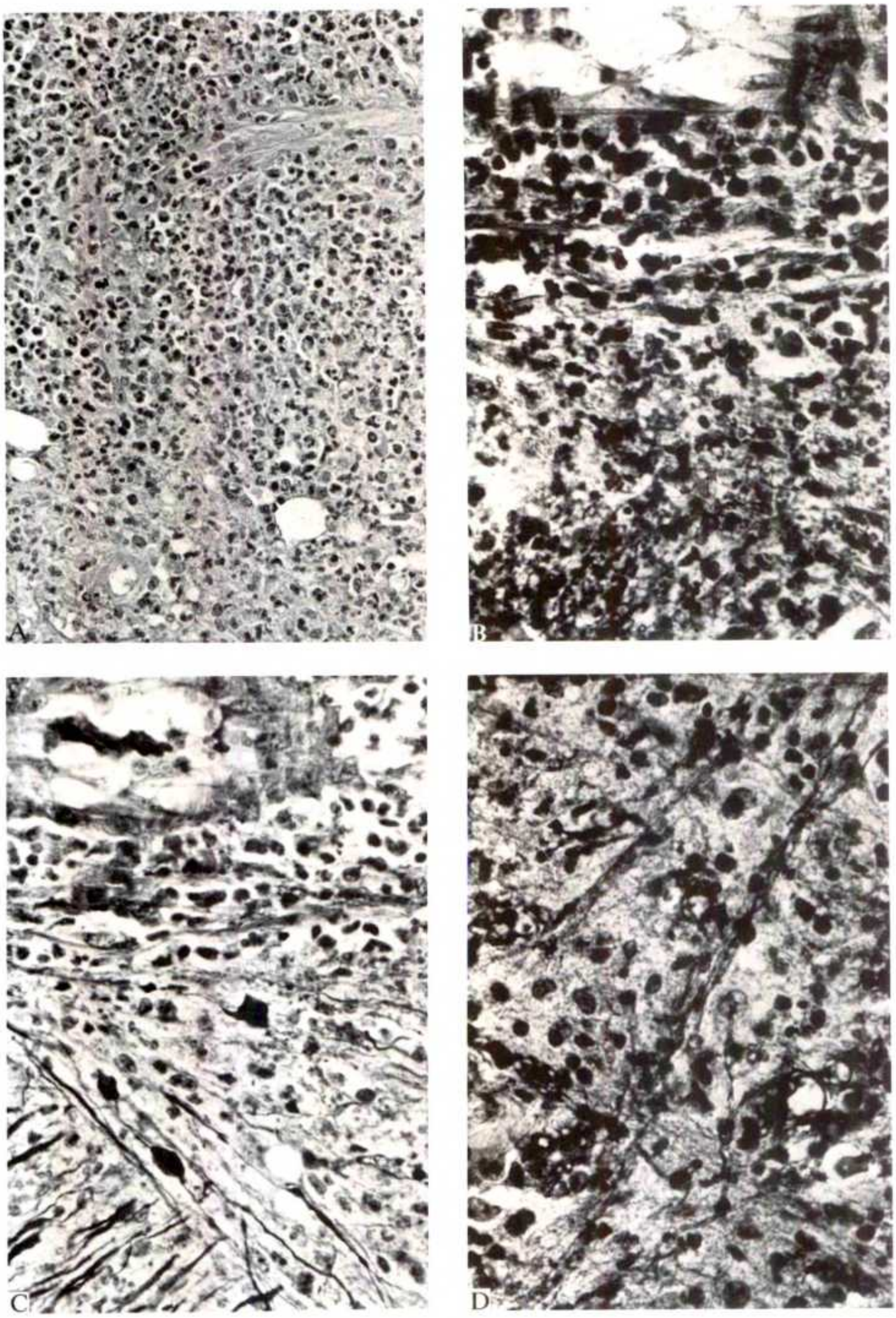


FIG. 3. Histopathology of lesion in *Case 1*. A, H and E (×330) showing diffusion of polymorphonuclear cells, lymphocytes and macrophages into tissue around the vessel. B, LFB-PAS-H (×530) showing LFB-positive myelin debris at the centre of the lesion. C, Holmes' stain (×530) showing swelling, beading and destruction of axons at the centre of the lesion. D, LFB-PAS-H (×530) showing swelling of myelin sheaths at the periphery of the lesion.

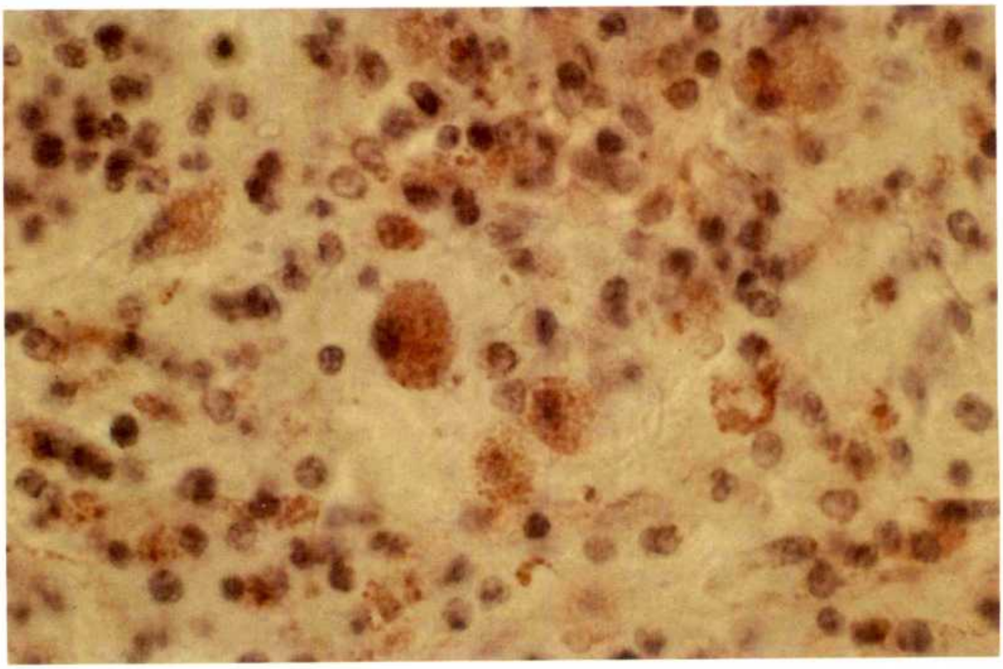


FIG. 4. ORO-H (×375) of frozen section showing macrophages filled with granular sudanphilic lipids.

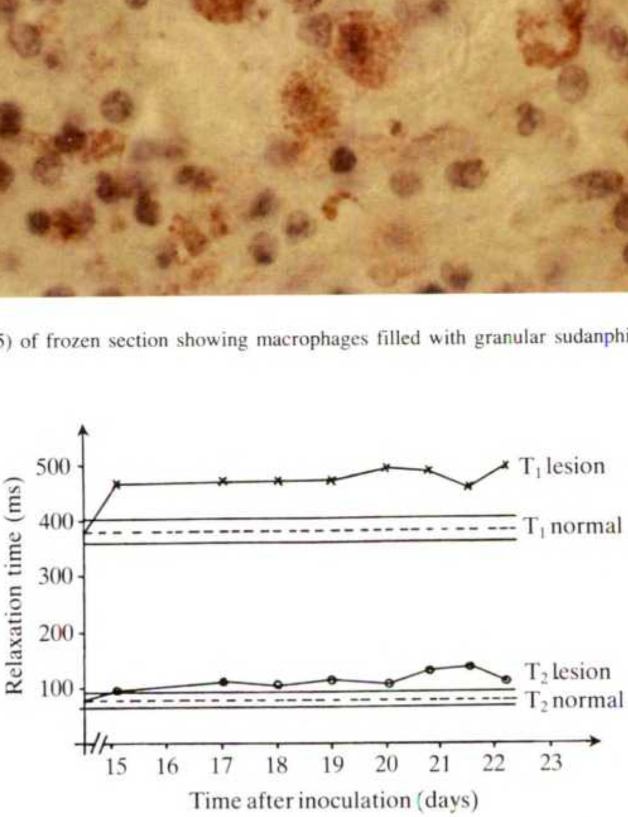


FIG. 5. Plot of T_1 and T_2 versus time after inoculation for *Case 1*. The dashed lines denote normal values of T_1 and T_2 ±2 SD.

Comparison of the MRI with the gross distribution of lesions. Photographs of the 5 mm thick brain section and the histological preparation were compared with the equivalent MRI. The left cerebral hemisphere lesion present on the MRI was seen clearly on the gross section (fig. 6B, C), as mildly haemorrhagic. In the histological preparation the lesion was 10×8 mm, centred around the genu of the internal capsule, extending slightly

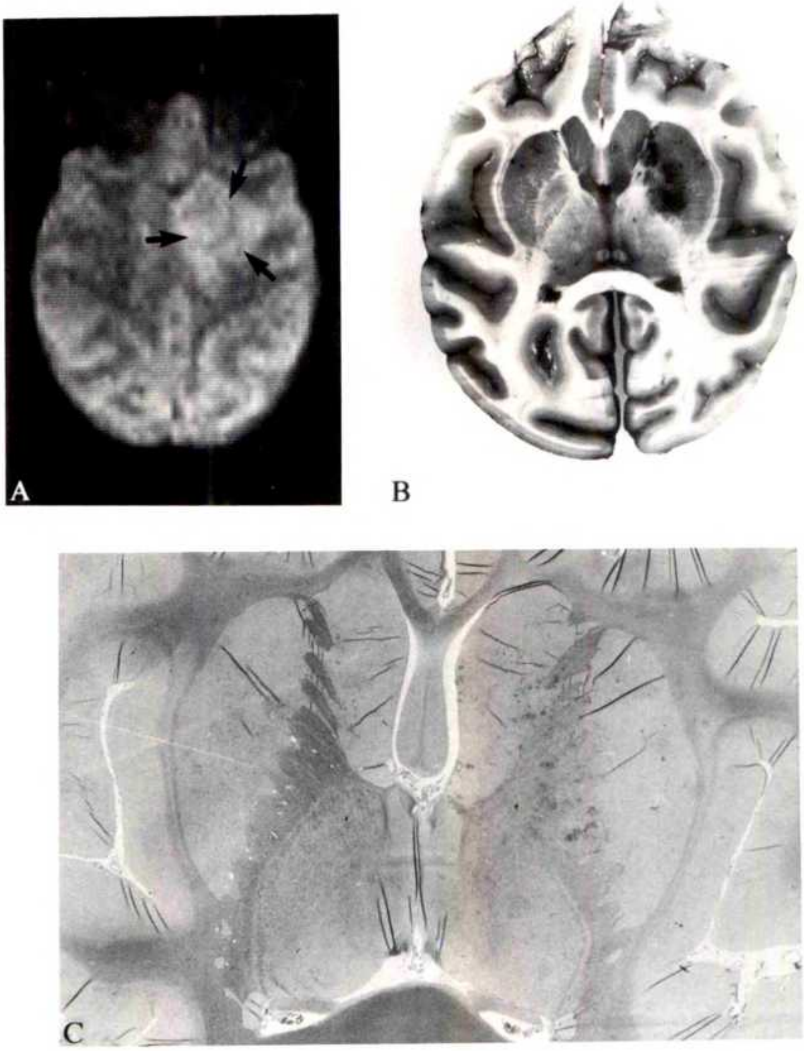


FIG. 6. Comparison of SE MRI with gross pathology for *Case 2*. A, $SE_{2000/40}$ MRI on day 22 after inoculation. The arrows show the extent of the lesion in the left cerebral hemisphere. B, inferior surface of 5 mm thick brain slice at the same level. C, LFB-PAS-H (×2) at the same level.

into the striatum (caudate and putamen) and into the anterior ventral nucleus of the thalamus. This lesion consisted of small areas of inflammation which did not coalesce.

Histology. The lesion had many of the same characteristics as the lesion in Case 1, but it was less confluent and was haemorrhagic. The medial half of the lesion consisted of smaller perivascular lesions (fig. 7A, B), while the lateral half consisted of larger

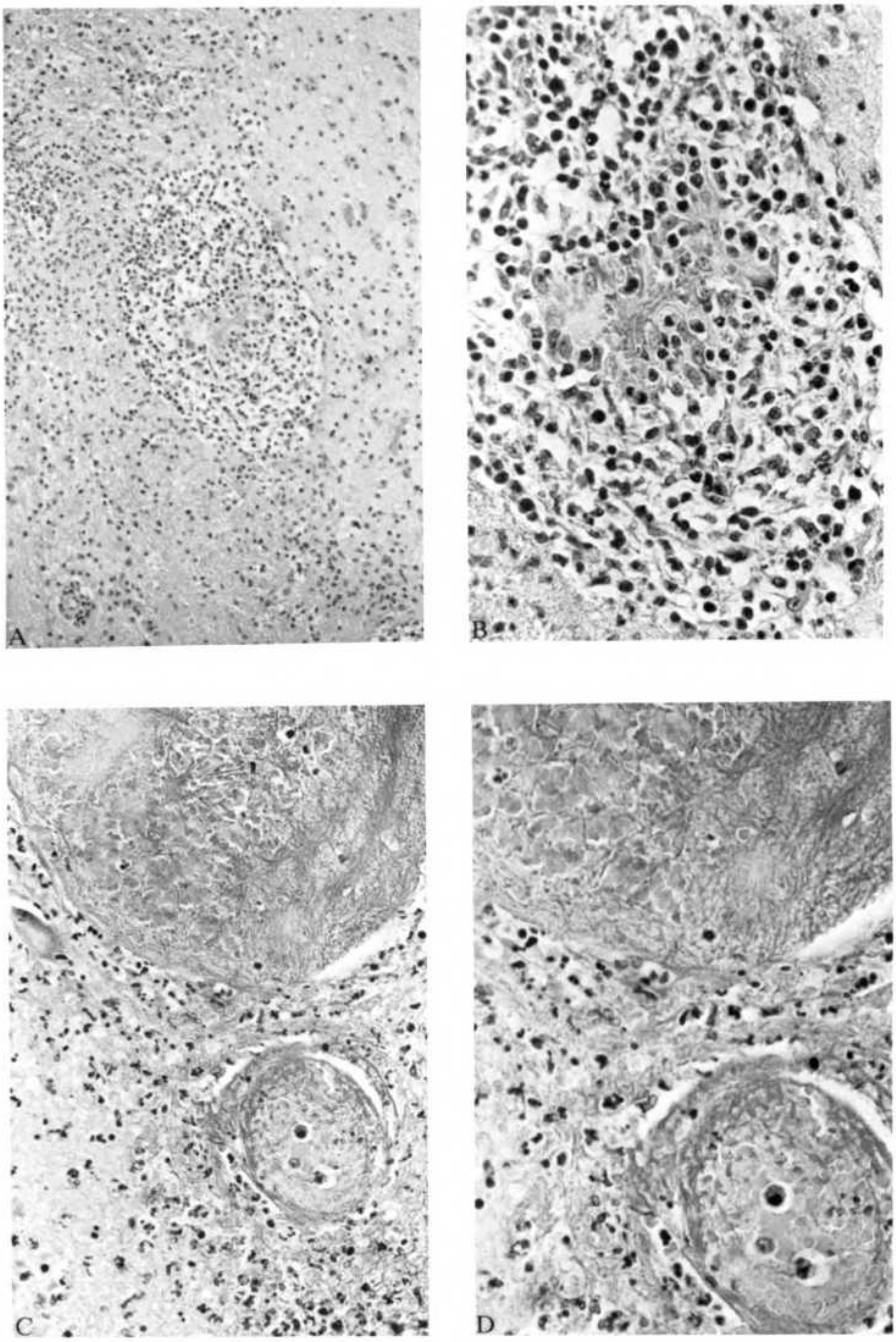


FIG. 7. Histopathology of lesion in *Case* 2. A, H and E (×130) of medial half of lesion. B, H and E (×330) of medial half of lesion. C, H and E (×330) of lateral half of lesion. D, H and E (×530) of lateral half of lesion.

haemorrhagic lesions (fig. 7C, D). Within this lesion there were collars of haemorrhage around the blood vessels, displacing the exudate peripherally. The exudate consisted mostly of polymorphonuclear leucocytes. A small amount of haemorrhage was seen in the anterior portion of the lesion, but the major component was posterior and lateral, and these two areas coalesced. At the site of thrombosed venules there were many polymorphonuclear cells. Some of the smaller lesions that comprised the large lesion seen grossly, had as many polymorphonuclear leucocytes but no haemorrhage; others were composed of predominantly mononuclear cells (lymphocytes and a few plasma cells) distending the Virchow-Robin spaces and infiltrating diffusely into the parenchyma.

Case 3

MRI and clinical observations. Areas of increased signal intensity on the SE images (TE = 40 ms and 60 ms) were seen in both cerebral hemispheres on day 11 after inoculation. There were no neurological signs observed at that time. Twelve hours later, the lesions in both hemispheres had enlarged (fig. 8A, B). The monkey's movements were slow and he was urinating frequently. A further 12 h later, the lesions had spread dramatically in both hemispheres. The monkey was clumsy and his peripheral vision was diminished. Seven hours later, he was found dead in his cage. A set of scans was obtained postmortem, and the brain removed and placed in 10% neutral buffered formalin. After 10 days of fixation, a set of scans was obtained from the fixed brain; the lesions were barely visible on the images of the fixed brain.

Comparison of the MRI with the gross distribution of lesions. Comparison of the photographs of the 5 mm thick brain sections and the histological preparations with the equivalent MRI showed that the lesions in both hemispheres corresponded well with the MRI appearance. At MRI level 3 (fig. 9A–D), 2 lesions could be seen. The left

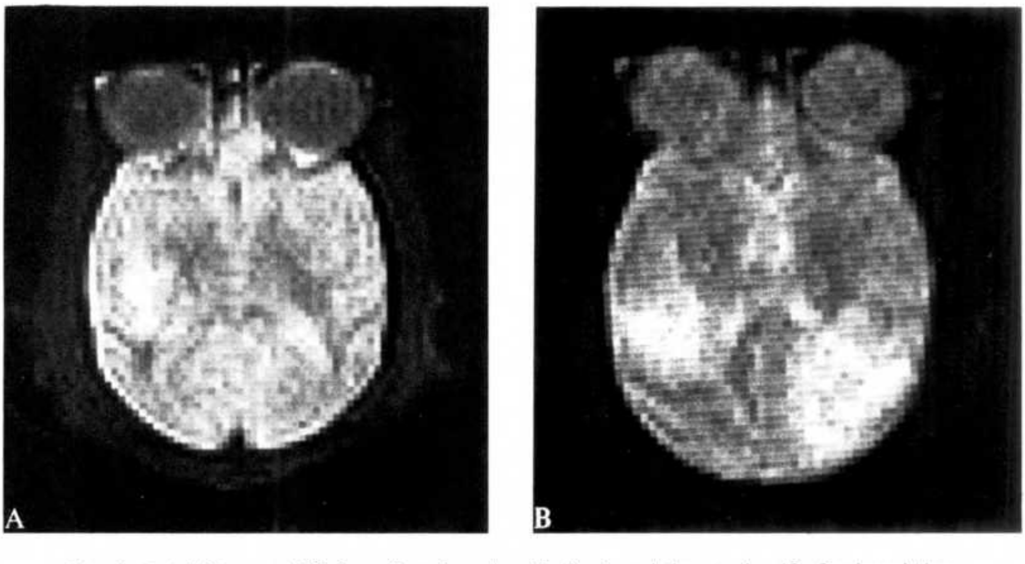


FIG. 8. Serial $SE_{2000/40}$ MRI from *Case 3*. A, day 11 after inoculation. B, day 12 after inoculation.

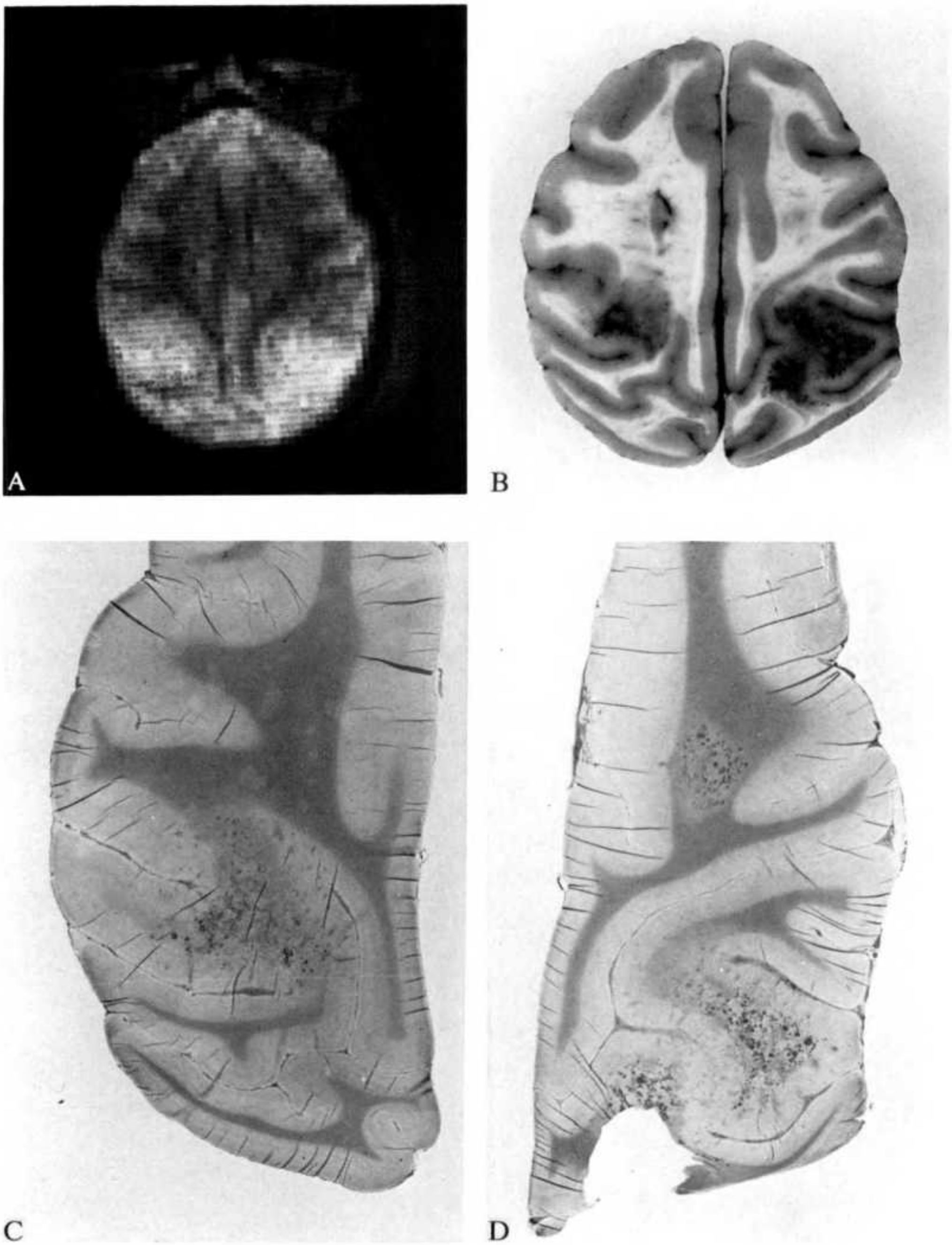


FIG. 9. Comparison of SE MRI with the gross pathology in *Case 3*. A, $\mathrm{SE}_{2000/40}$ MRI level 3. B, inferior surface of 5 mm brain slice at the same level. C, Gallo-DR (×2) of right cerebral hemisphere. D, Gallo-DR (×2) of left cerebral hemisphere.

cerebral hemisphere lesion was present in the white and grey matter of the occipital lobe, and measured 12 × 15 mm. The right-sided lesion was more anterior, and present predominantly in the white matter; it measured 12 × 11 mm. At lower levels the lesions were larger. The left cerebral lesion involved the temporal cortex and white matter, and measured 17 × 10 mm. The lesion included the choroid plexus of the trigone and extended to the posterior edge of the putamen. Isolated perivascular lesions could also be seen at the level of the anterior commissure and beneath the ependyma overlying the head of the caudate.

The right cerebral lesion measured 18 × 12 mm and involved the lateral edge of the thalamus (lateral geniculate and pulvinar), the posterior edge of the globus pallidus and the posterior half to two-thirds of the putamen; it also included the external and extreme capsules and the anterior portion of the visual radiation.

Histology. In MRI no. 3, corresponding to brain slice 2 (fig. 9A, B) there were 2 lesions, 1 in each hemisphere. The left lesion (fig. 9D) consisted of 2 areas: posteriorly the lesion was more haemorrhagic, being diffusely so in the white matter; the haemorrhage occurred around small vessels that were only slightly inflamed. The lesions in the white matter were large compared with those in the cortex, which were not haemorrhagic. The exudate was predominantly polymorphonuclear cells. The anterior portion of the lesion was mostly present in the white matter and it was similar to the posterior portion with a large exudate mainly containing polymorphonuclear cells. The lesion in the left cerebral hemisphere was a mixture of haemorrhagic and nonhaemorrhagic areas (fig. 10A). The haemorrhagic areas were found on the periphery of the lesion with very little inflammation (fig. 10B); in the centre of the lesion there were areas of perivascular inflammation with exudate similar to that in Cases 1 and 2 (fig. 10C, D). The right cerebral lesion in MRI no. 3, corresponding to brain slice 2 (fig. 9C) contained many axons which showed swelling and fragmentation. A large number of axons remained intact in spite of the large size and compactness of the exudate (fig. 11A–C). In the larger lesions within this area the axons were almost completely destroyed. There was no sudanophilic debris observed in this lesion. The haemorrhagic areas were only present in the white matter.

The lesions in Case 3 were more haemorrhagic and necrotic than the lesions in Cases 1 and 2. In addition, the areas of inflammation and haemorrhage were more confluent than in Cases 1 and 2.

Quantitative MRI. The T_1 and T_2 values of the left hemisphere lesion in MRI slice 4 were measured as a function of time after inoculation and were shown to increase from the time of detection until death. The increase was not dramatic (fig. 12).

Case 4

MRI and clinical observations. An area of increased signal intensity on the SE image was observed in the left cerebral hemisphere on day 15 (Stewart *et al.*, 1985) after inoculation (fig. 13). This lesion was not associated with any neurological signs, apart from a small loss in weight. The lesion spread laterally, medially, superiorly and inferiorly over the course of the next 3 days (fig. 14A–C). On day 17 after inoculation, the monkey appeared spastic in his right arm which became progressively more severe. Later the

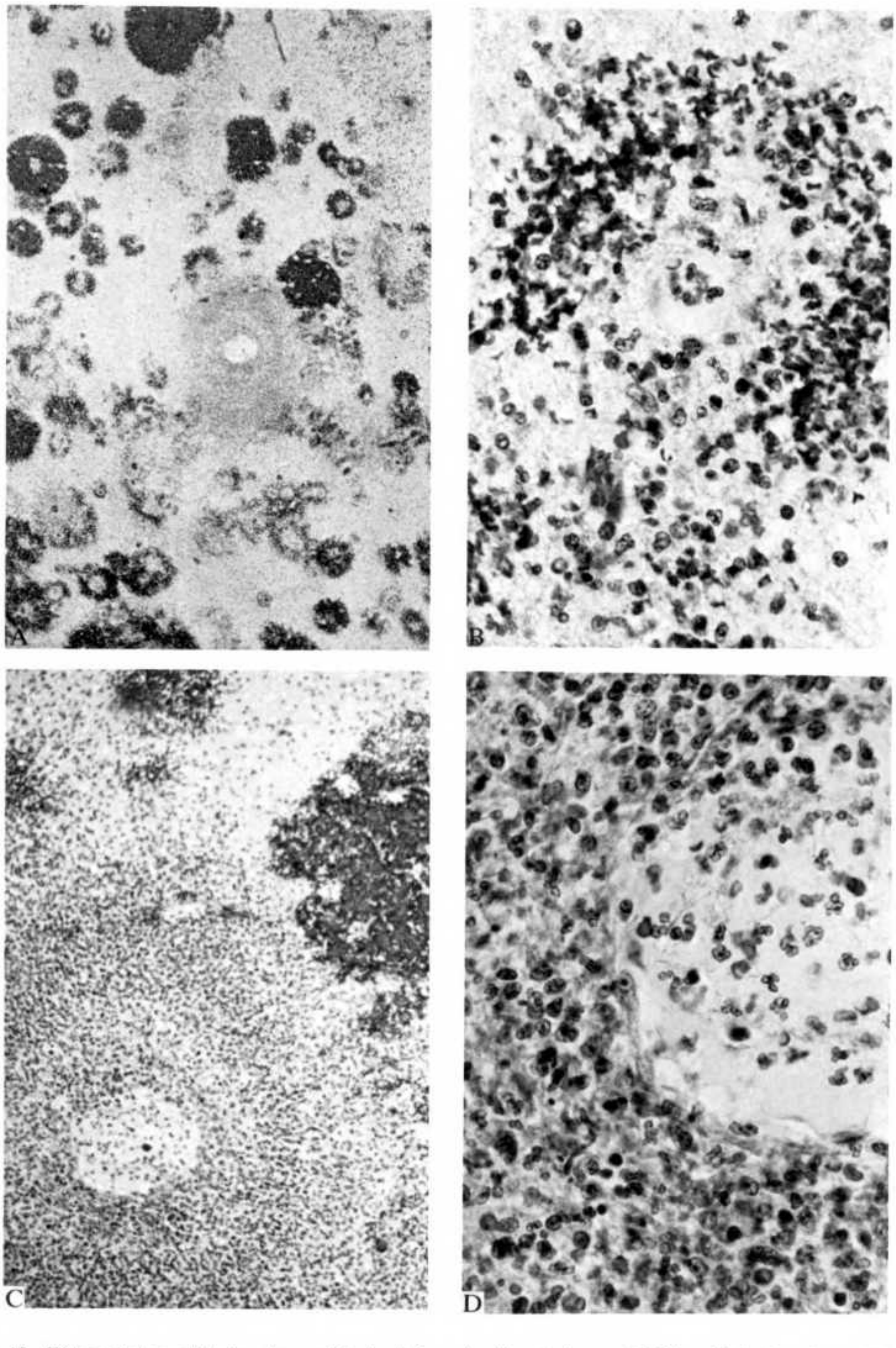


FIG. 10. Histopathology of lesion observed in the left cerebral hemisphere at MRI level 3 in *Case 3*. A, Gallo-DR (×33) showing haemorrhagic and nonhaemorrhagic areas. B, Gallo-DR (×530) showing haemorrhage with very little inflammation. C, Gallo-DR (×130) showing perivascular inflammation. D, Gallo-DR (×530) showing exudate similar to that in Cases 1 and 2.

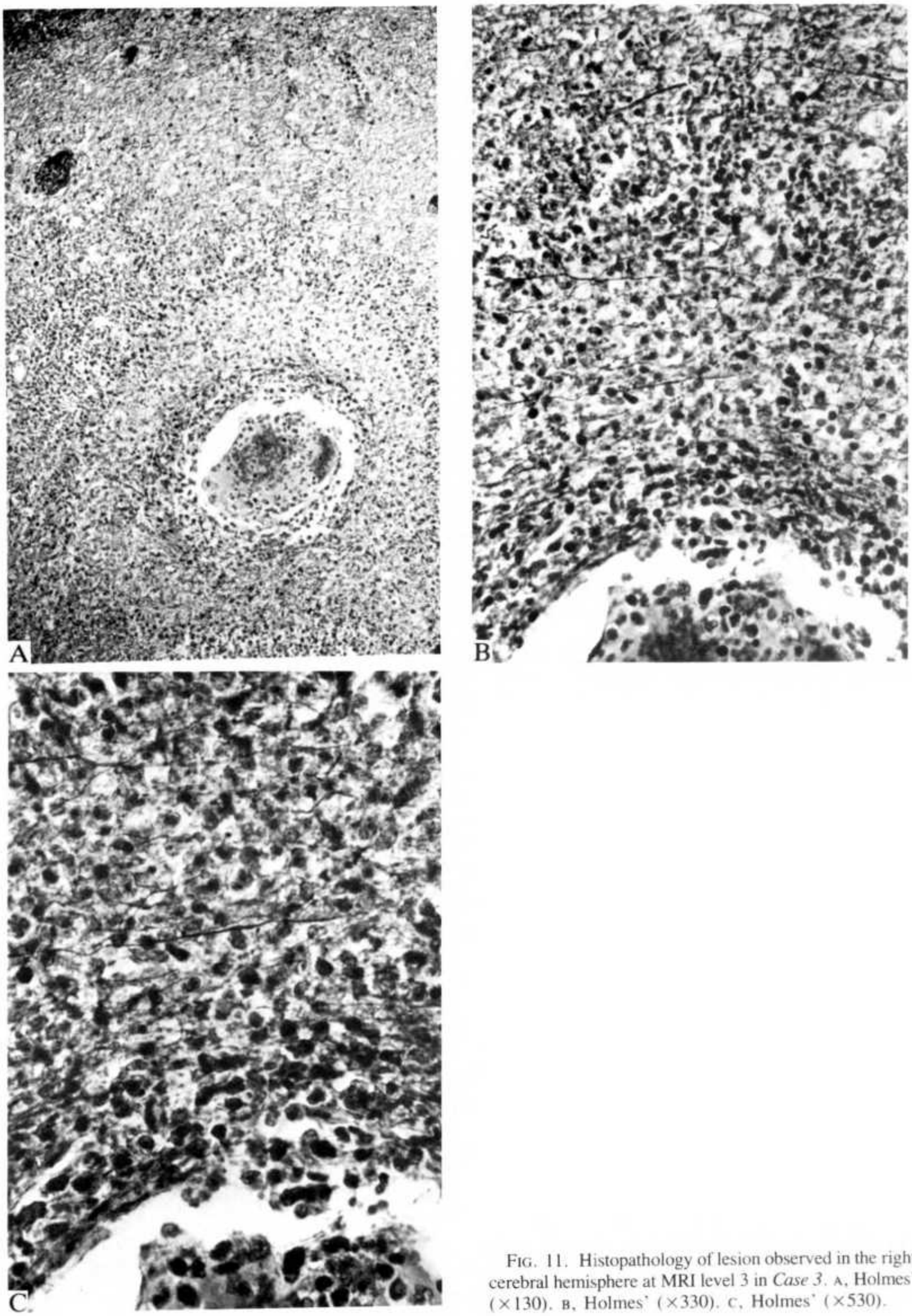


FIG. 11. Histopathology of lesion observed in the right cerebral hemisphere at MRI level 3 in *Case 3*. A, Holmes' (×130). B, Holmes' (×330). C, Holmes' (×530).

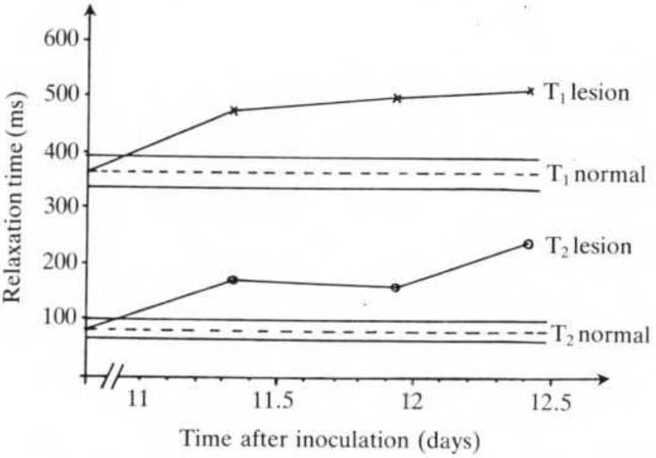


FIG. 12. *Case 3*. Plot of T_1 and T_2 versus time after inoculation for the left cerebral hemisphere lesion in slice 4. The dashed lines denote normal values of T_1 and T_2 ±2 SD.

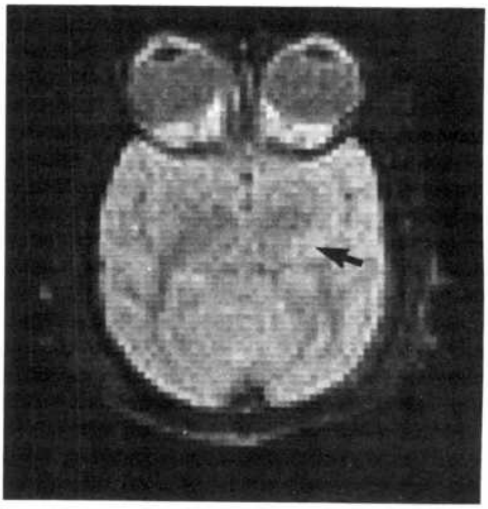


FIG. 13. $SE_{2000/40}$ MRI obtained from *Case 4* on day 15 after inoculation. The arrow indicates the lesion in the left cerebral hemisphere.

same day he seemed to neglect his right visual field, suggesting a right hemianopia; on day 18 his left leg was affected.

Twenty-four hours later he was prostrate in his cage and his whole right side appeared spastic. He was killed at 2 a.m. on day 20 after inoculation. An immediate postmortem scan was obtained, and the brain was removed and placed in 10% neutral buffered formalin. Only parts of the lesion could be seen on the MRI of the fixed brain (fig. 15).

Comparison of the MRI with the gross distribution of lesions. Comparison of photographs of the 5 mm thick brain sections and the histological preparations with the equivalent MRI, showed a large haemorrhagic lesion in the left cerebral hemisphere

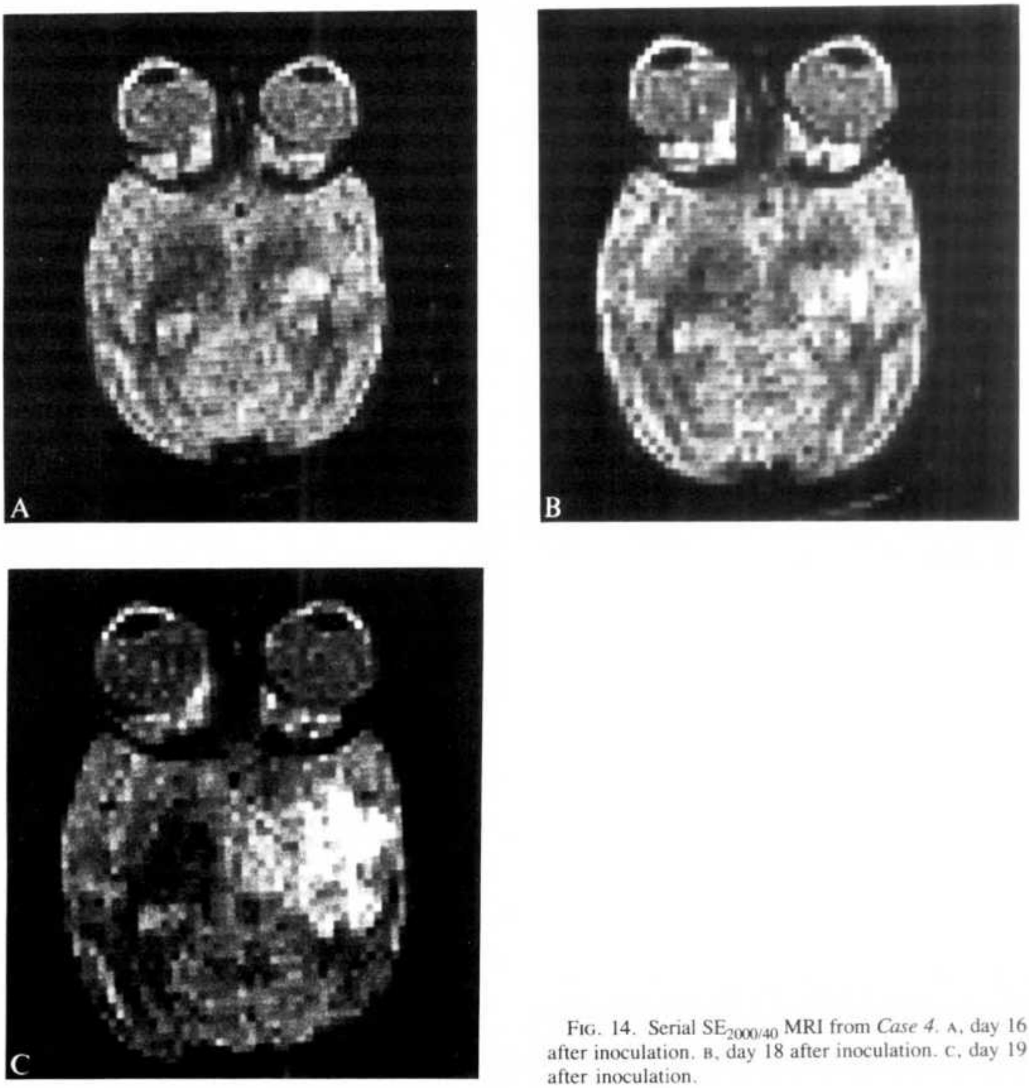


FIG. 14. Serial $SE_{2000/40}$ MRI from *Case 4*. A, day 16 after inoculation. B, day 18 after inoculation. C, day 19 after inoculation.

(fig. 16A–C). The lesion measured 21 × 18 mm and involved the genu and posterior limb of the interior capsule, the posterior two-thirds of the putamen, the external and extreme capsule and the insular cortex. It also involved the temporal cortex and white matter anteriorly and extended back to the anterior portion of the visual radiation, the tail of the caudate and the lateral thalamus (lamina medullaris or internus thalami). Smaller inflammatory lesions were also seen in the splenium of the corpus callosum, the columns of the fornix and in the right anterior thalamic nucleus. Smaller subependymal lesions were also present at the head of the caudate on the left side. At lower levels the lesion also involved the lateral geniculate nucleus, the optic tract and the lateral half of the amygdaloid nucleus.

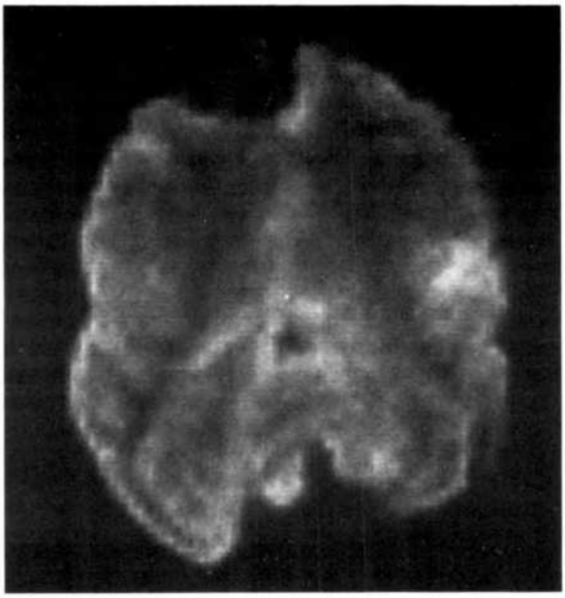


FIG. 15. $SE_{2000/40}$ MRI of fixed brain from *Case 4* showing abnormal area in left cerebral hemisphere.

A smaller lesion, measuring 6×3 mm, was present in the right cerebral hemisphere; it involved the retrolenticular part of the internal capsule and was visible on the MRI.

Histology. The larger lesion in the left cerebral hemisphere was quite oedematous, necrotic and haemorrhagic laterally and anteriorly (fig. 17A, B) with extensive destruction of axons. More posteriorly and medially, involving the thalamus, it was only slightly haemorrhagic.

The most posterior part of the lesion involving the visual radiation (fig. 18A) was more demyelinated than necrotic, with abundant myelin debris, many polymorphonuclear cells and lymphocytes (fig. 18B). There was almost complete preservation of axons with only a few axon swellings (fig. 18C). An old infarct could also be seen in the anterior temporal lobe and cortex; it showed gliosis with wallerian degeneration of the underlying white matter, and only slight inflammation. This old infarct was the region that could be clearly seen on the images of the fixed brain (fig. 15).

In the corpus callosum (fig. 19A), the exudate displaced most of the axons to the periphery (fig. 19B), but a few myelinated axons coursed through the exudate (fig. 19C, D).

The smaller lesion in the right hemisphere showed moderate destruction and displacement of axons, but many myelinated and demyelinated axons coursed through the exudate. The exudate was made up of predominantly polymorphonuclear cells with a large number of lymphocytes and histiocytes.

Quantitative MRI. The T_1 and T_2 values of the lesion first observed on day 15 were measured as a function of time after inoculation (fig. 20); both sets of values increased from the day of detection until death.

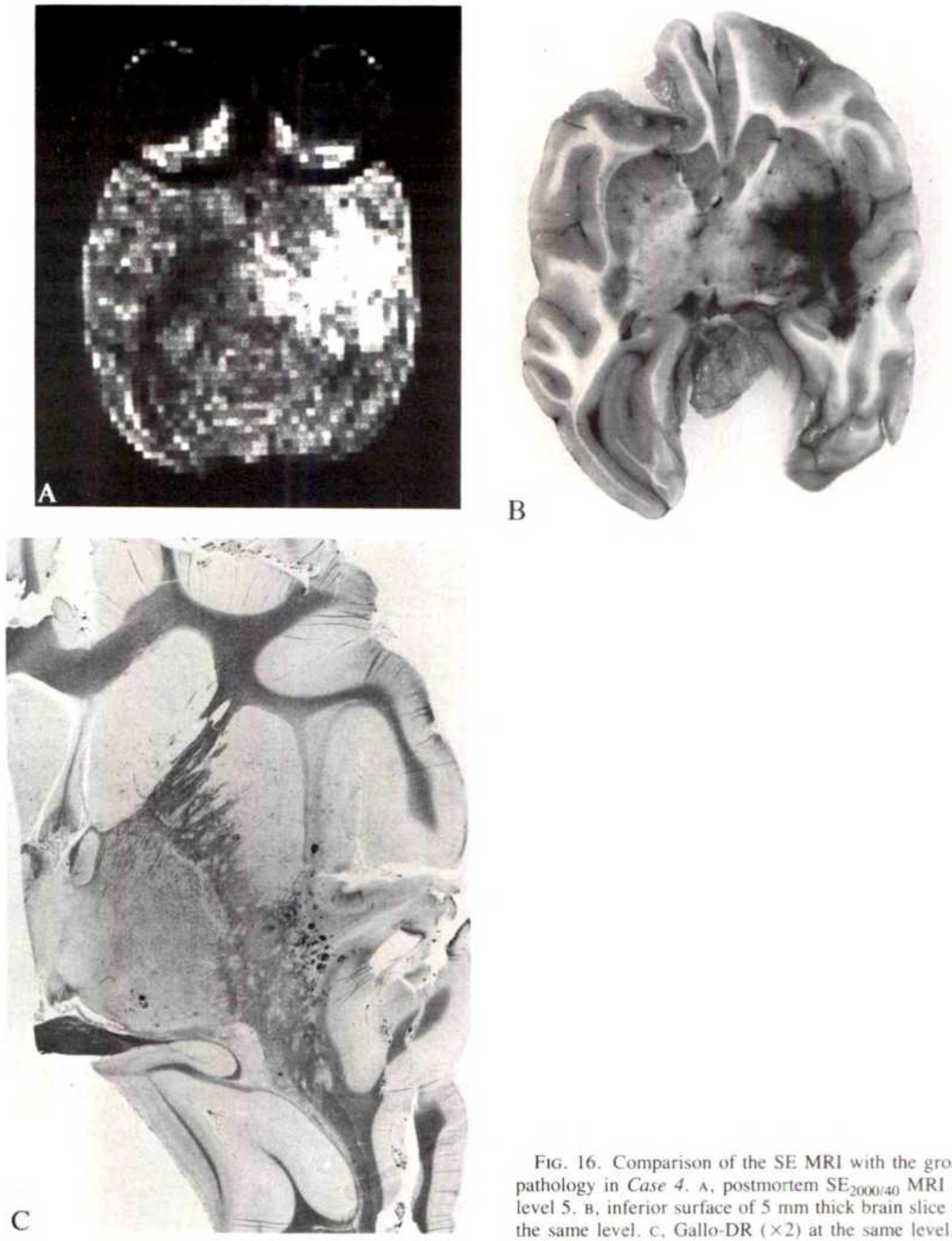


FIG. 16. Comparison of the SE MRI with the gross pathology in *Case 4*. A, postmortem SE$_{2000/40}$ MRI at level 5. B, inferior surface of 5 mm thick brain slice at the same level. C, Gallo-DR (×2) at the same level.

In each of the 4 cases presented above, the same lesion was followed over time to provide each plot of T_1 and T_2 versus time after inoculation. The T_1 and T_2 values of these lesions at the time of death are given in Table 2 in order of increasing T_1 and

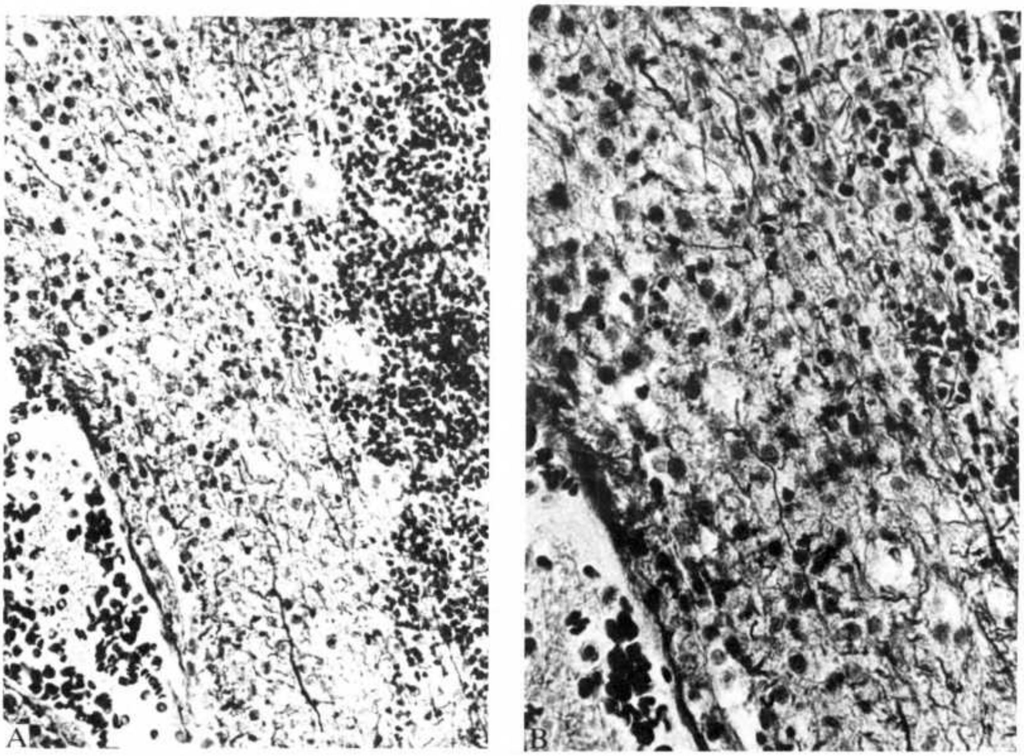


FIG. 17. Histopathology of anterior and lateral part of lesion in the left internal capsule of *Case 4*. A, Holmes' (×330). B, Holmes' (×530).

T_2, which also corresponds to an increase in the degree of inflammation, demyelination and haemorrhagic necrosis.

Summary of results

1. MRI detected acute EAE lesions in primates when only prodromal signs were present clinically. This MRI change was due to an increase in the T_1 and T_2 values in the lesion area. Preclinical lesions have also been seen in the CNS of guinea pigs with EAE (O'Brien *et al.*, 1987).
2. MRI was an accurate indicator of the gross extent of the lesions of acute EAE.
3. The oldest lesions were the most haemorrhagic, but the haemorrhage occurred in two patterns, one near vessels displacing the exudate peripherally and one at a slight distance from a much less inflamed vessel. Only the former pattern is consistent with the classical belief that haemorrhage is a secondary event in EAE.
4. In lesions with varied histological components the MR characteristics of the lesions also varied (Table 2). Conversely, microscopically similar lesions had the same MR characteristics.
5. As the disease progressed, the MRI characteristics also changed. Since the T_1 and

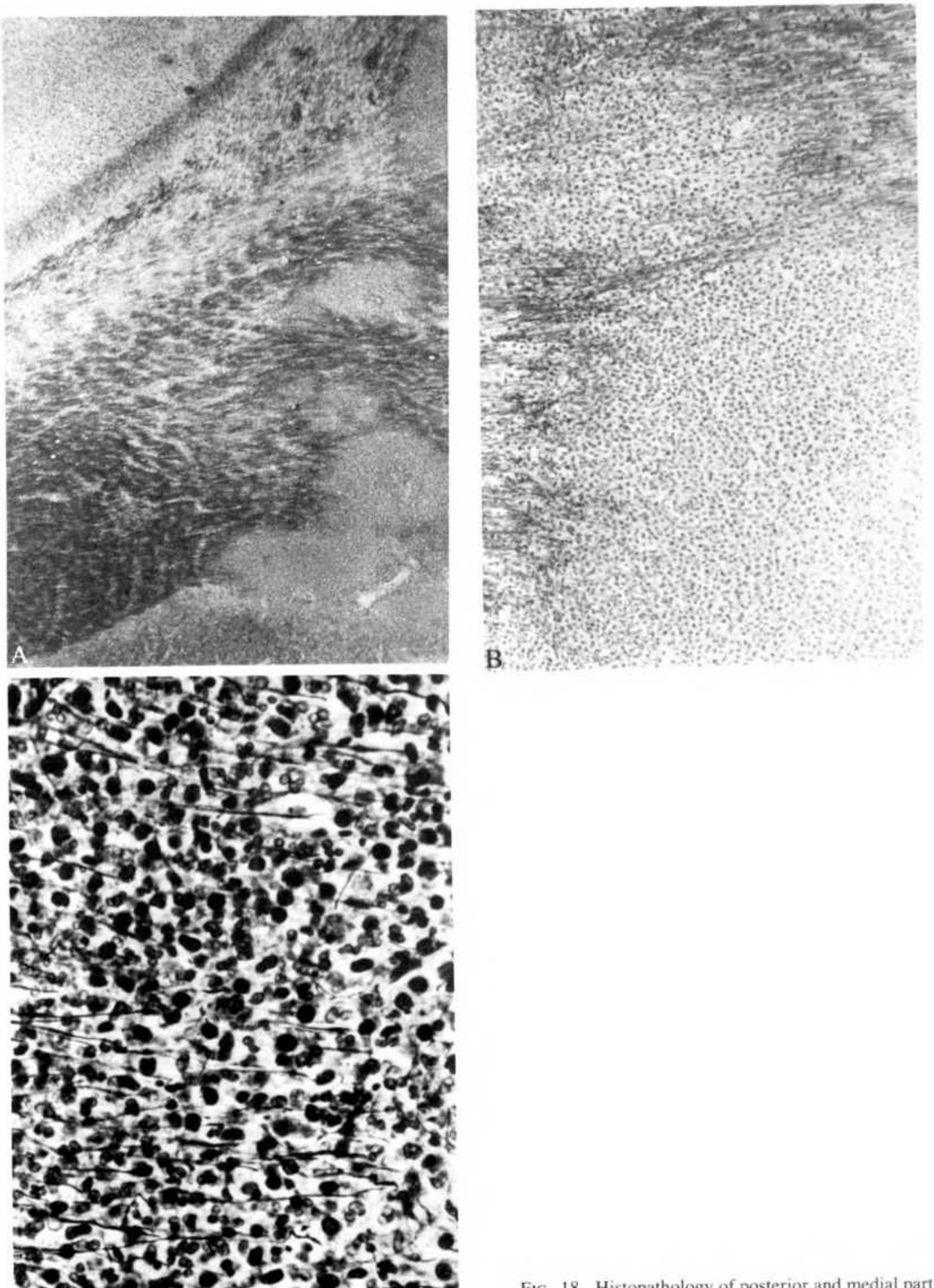


FIG. 18. Histopathology of posterior and medial part of lesion in the left visual radiation of *Case 4*. A, LFB-N (×33). B, LFB-N (×130). C, Holmes' (×530).

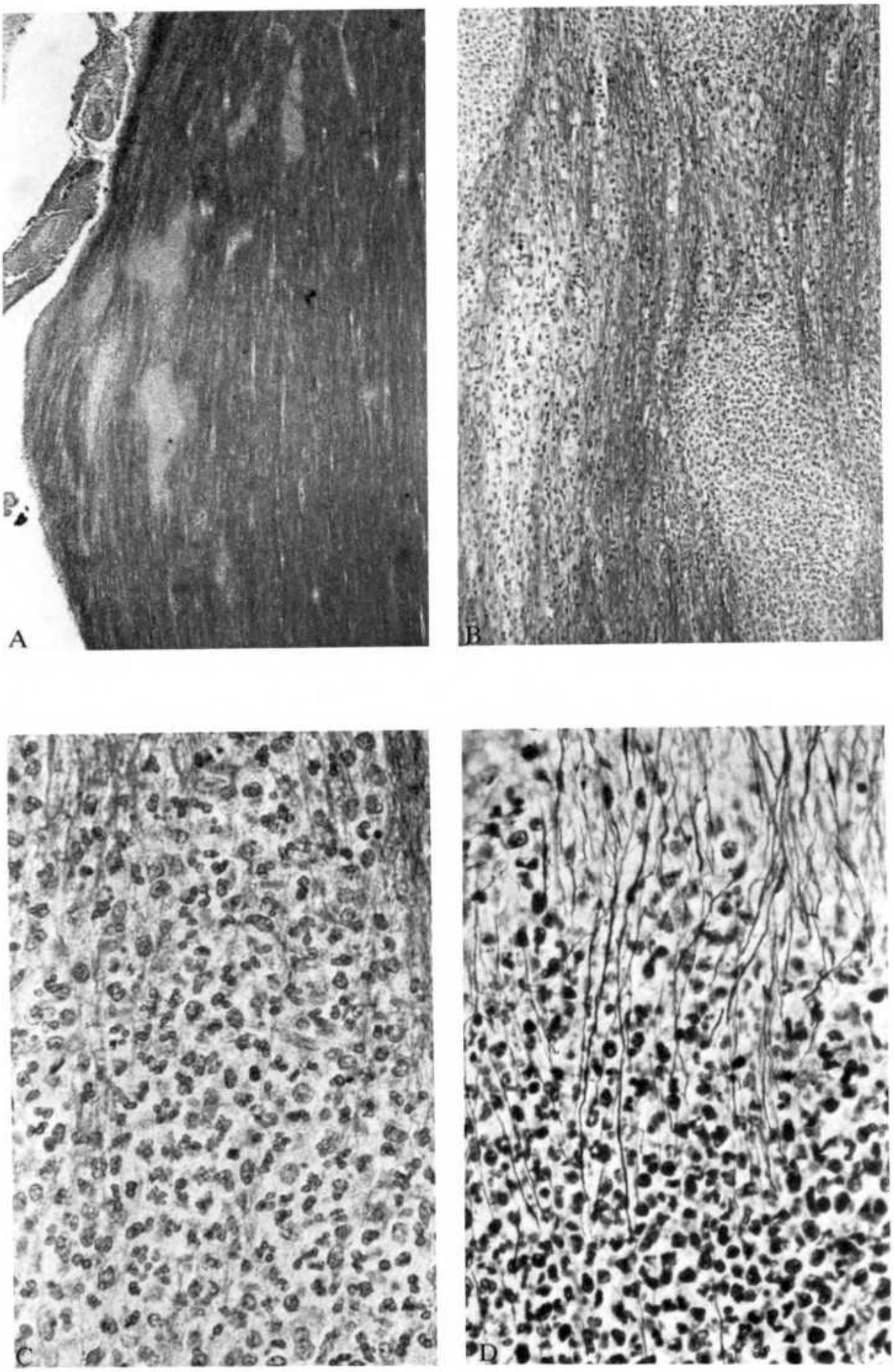


FIG. 19. Histopathology of lesion in the corpus callosum of *Case 4*. A, LFB-N (×33). B, LFB-N (×130). C, LFB-N (×530). D, Holmes' (×530).

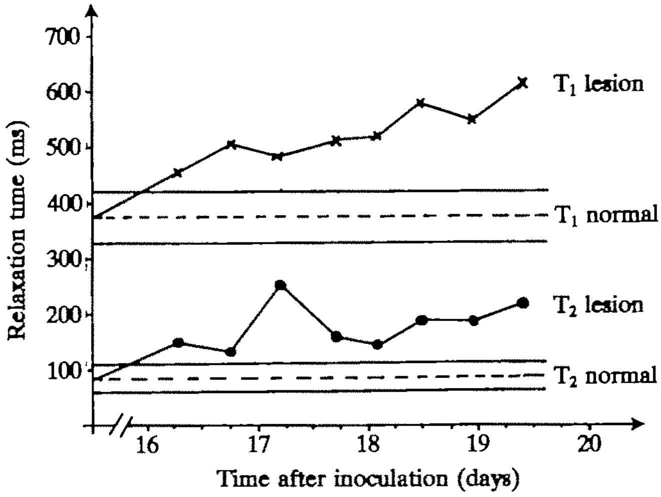


FIG 20. Plot of T_1 and T_2 versus time after inoculation from lesion first seen on day 15 after inoculation in *Case 4*. The dashed lines denote normal values of T_1 and T_2 ±2 SD.

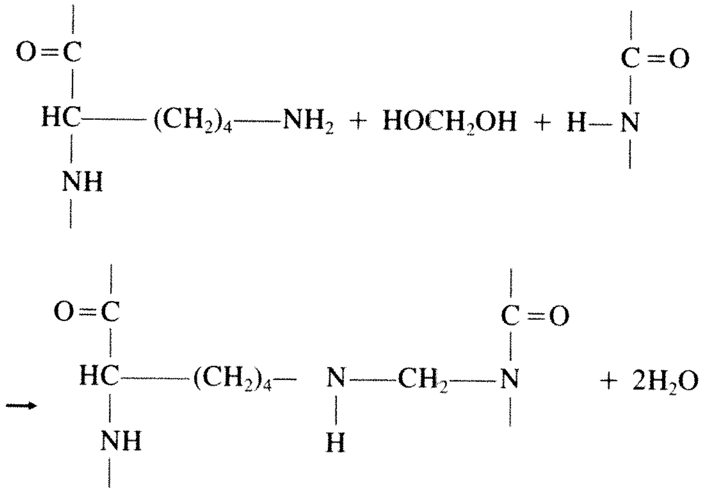
T_2 values are sensitive to the molecular environment around the protons, these MRI changes are indicative of progressive lesion evolution at a molecular level.

6. Acute inflammatory lesions could not be seen on MRI of the fixed brain. Formalin fixation of these lesions gives rise to a molecular environment that is indistinguishable from normal tissue using MRI. However, just as we have seen in MS tissue (Stewart *et al.*, 1986), the small gliotic lesion in Case 4 could be seen in the images of the fixed brain. The inability to clearly define inflammatory lesions on the fixed brain images is being further investigated.

DISCUSSION

The present study shows that MRI can detect EAE in primates before the onset of neurological signs; similarly, in MS, MRI-visible lesions may appear in the CNS unaccompanied by any new clinical symptoms. It remains to be proved whether the first lesion in EAE is pathologically similar to the clinically asymptomatic lesions in MS. It is recognized that haemorrhage is not frequently seen in the CNS of MS patients; however, it may be that the perivascular inflammatory lesions, which may or may not be confluent, could be similar in both acute EAE and MS. Serial MRI studies carried out on MS patients at the University of British Columbia (UBC) (Isaac *et al.*, 1988; Koopmans *et al.*, 1989; Willoughby *et al.*, 1989) have shown asymptomatic MRI detected lesions to come and go with a frequency that is much higher than the development of clinical signs; most of these lesions become smaller and some disappear with time. The time relations make it unlikely that these new and disappearing lesions are demyelinating; it is likely that they are primarily inflammatory. One must be wary of assuming a complete recovery of these areas of the brain, since the disappearance of a lesion on the MRI does not necessarily indicate that the tissue has returned to normal.

It has been interesting to note that inflammatory lesions are either barely visible or not visible at all on the MRI of fixed tissues. Formaldehyde fixation of tissues causes the formation of methylene bridges between the ϵ-amino group of lysine and available amide groups in peptide linkages of other proteins (Kiernan, 1981). The proteins present in the exudate of the inflammatory lesions will be cross-linked on fixation, which must give rise to a relationship between bound and free water similar to that seen in normal fixed tissue. However, fixed demyelinated and gliotic lesions are both visible on MRI (Stewart *et al.*, 1986; Nagara *et al.*, 1987). In vitro quantitative MR studies on fresh and fixed tissue are in progress (Stewart *et al.*, 1988) and it is anticipated that these studies should help us to understand more clearly the mechanisms giving rise to the changes in T_1 and T_2. The areas of abnormality seen grossly on the fixed brain slices in EAE correspond well with the areas of increased signal intensity seen on the SE MRI. This finding has important implications when studying the evolution of the pathological process over time in both EAE and MS. If MRI is to be used to measure the extent of disease, it must be both accurate and reproducible. Repositioning of the subject in follow-up studies is critical. For some of the lesions observed in the primates, the MRI abnormalities were more extensive than that seen macroscopically; this appears to be due to the effects of volume-averaging, since we are comparing the surface of a brain slice with an MR image obtained from a 5 mm slice thickness. Varying the imaging parameters also affects the size and conspicuousness of lesions (Posin *et al.*, 1985). However, for the TE and TR values used in these studies this is not a problem. Studies in MS patients at UBC (using a truncated sinc-shaped radio-frequency pulse to excite a 10 mm thick slice), have shown that a 3 mm deliberate offset in the slice position causes an overall change in lesion area of 11%; changes in lesion area for individual slices with smaller lesions can be as high as 50% (Isaac *et al.*, 1988). Repositioning is also critical for the quantitative measurement of MRI parameters. The images of the monkeys were obtained by selectively exciting a slice 5 mm thick. Within this thickness there may be water protons in different compartments or in different types



$$\mathrm{\underset{\underset{|}{NH}}{\overset{\overset{|}{O=C}}{HC}}{-}(CH_2)_4{-}NH_2 + HOCH_2OH + H{-}\underset{|}{\overset{\overset{|}{C=O}}{N}}}$$

$$\rightarrow \mathrm{\underset{\underset{|}{NH}}{\overset{\overset{|}{O=C}}{HC}}{-}(CH_2)_4{-}\underset{H}{\underset{|}{N}}{-}CH_2{-}\underset{|}{\overset{\overset{|}{C=O}}{N}}} + 2H_2O$$

of tissue which are not rapidly exchanging; this may give rise to multiexponential relaxation (Bakker and Vriend, 1984; Kamman *et al.*, 1987). The signal contribution from each proton source depends on the proton density and on the pulse sequence used to excite the slice and the resulting image intensity in each pixel is the sum of the signal contributions through the slice. This means that the computed images can only provide observed T_1 and T_2 values, giving no indication as to their source. Accurate repositioning is essential, since variations in T_1 and T_2 may be due to variations in the contributions to the signal from different compartments within the slice. In addition, the lesion may increase in size, filling the slice: this gives rise to an 'observed' increase in the relaxation times because of the increased contribution to the signal from the abnormal area. In this study, in order to minimize the effects of volume-averaging described above, quantitative measurements were obtained from the centre of the lesions and, where possible, from a lesion that extended through more than one slice.

The results of our quantitative studies show that the T_1 and T_2 values increase over time while the disease was evolving; when clinical signs were stable, the T_1 and T_2 values also stabilized. This finding seems to indicate that the pathological progression of the disease also halted, since T_1 and T_2 are sensitive to changes in the molecular environment. It must also be noted, however, that small changes in T_1 and T_2 ($\leq 10\%$) will not be observed, and some molecular changes may cancel each other out. When studying the development of EAE lesions in Case 4, we noted that T_1 began to increase before the lesion became visible on the SE image. This change has also been seen in other cases of EAE in primates, and indicates that the early pathological changes affect T_1 rather than T_2. However, the increase in T_1 is small, and falls within the error boundaries of the measurement; therefore although reproducible, the T_1 change was not statistically significant.

In order to use T_1 and T_2 to distinguish between different types of lesions, the changes in the measurements must be large compared with the errors involved. Of the 4 EAE cases presented here, the most dramatic changes in T_1 and T_2 were observed in Case 4, corresponding to lesions that were more demyelinated and haemorrhagic than those in the other 3 cases. MRI studies on intracranial haemorrhage (Doomis *et al.*, 1986) have shown that the T_1 and T_2 values from subacute and chronic haemorrhage (greater than 3 days old) are lower than those obtained from acute haemorrhage (less than 3 days old). This is thought to be due to the oxidation of haemoglobin to methemoglobin, which is paramagnetic. This occurs after about 3–4 days (Koenig *et al.*, 1981; Bradley and Schmidt, 1984). In Case 4, the lesion was more than 4 days old, therefore the contribution to the observed T_1 and T_2 values due to haemorrhage will be lower than in Cases 2 and 3, which are considered acute haemorrhage. The T_1 and T_2 values are still greatest for Case 4, confirming that haemorrhage is not solely responsible for the observed changes in these parameters. At present, the influence of demyelination on the relaxation of tissue water protons is not known. From our studies on human tissue, we know that demyelination causes an increase in T_1 and T_2 but how this compares with the effects of inflammation has not yet been established. The difference in T_1 between a lesion with no haemorrhage and little demyelination (Case 1) and the lesions of Case 4 is 100 ms. This difference is large enough to be considered significant. Table 2 shows that the relaxation times for Case 2 are lower than might be expected, based on the type of pathology that was present. These low values can be explained in terms of volume averaging. The lesion in this case consisted of small inflammatory areas which did not coalesce. The area of increased signal intensity on the SE image is therefore not homogeneous, and any area chosen to obtain values for the relaxation times will consist of normal and abnormal tissue, giving rise to the lower T_1 and T_2 values. At the present time, the pathological process primarily responsible for the difference in MRI parameters in the lesions seen in these 4 cases cannot be identified. Further studies on demyelinating lesions will be necessary before we can state which pathological process gives rise to the different changes in T_1 and T_2. From our postmortem MS studies, we consider that the most gliotic lesions have the highest T_1 values (Stewart *et al.*, 1986). In addition, we found the T_1 values in a case with large areas of partial demyelination and gliosis to be different from those obtained from gliotic lesions that were totally demyelinated. Where inflammation fits into this picture is not yet understood. Both inflammatory and gliotic lesions are associated with an increase in water content, which in turn affects the relaxation parameters. It may be that inflammation and gliosis will have similar MRI characteristics; they could, however, be distinguished using the length of time they have been present in the brain.

TABLE 2 IMMEDIATE POSTMORTEM T_1 AND T_2 VALUES FROM LESIONS FOLLOWED OVER TIME IN CASES 1–4*

Case	*T_1 (ms)*	*T_2 (ms)*
1	500	120
2	460	110
3	540	260
4	600	270

* These numbers were obtained by outlining a region of interest on computed T_1 and T_2 images.

In following MS, MRI allows repeated study of the same patient without causing any known damage to the tissues; accordingly MRI has already proved to be very useful in the diagnosis of MS. We feel that it has even greater promise of demonstrating the evolution of the pathological process so that it can be used to follow the course of the disease. Such disease monitoring could be aided by using quantitative MRI to distinguish acute inflammatory lesions from more chronic demyelinating ones. Several technical improvements would help to this end; by working at higher field strength, with the associated increase in signal-to-noise, it is possible to obtain substantially higher in-plane resolution and to excite thinner slices, both of which decrease the effects of volume averaging.

ACKNOWLEDGEMENTS

We wish to thank the Medical Research Council of Canada for the grants awarded to D.W.P. and L.D.H. in support of this work. Thanks also go to the National Science and Engineering Research Council for the grant awarded to L.D.H. We are grateful to R. Koopmans and C. Isaac for their help with the animals, and to the Animal Resource Facility at the University of British Columbia for their cooperation throughout this project.

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(Received June 7, 1989. Revised October 9, 1990. Accepted October 19, 1990)

Brain (1991), **114**, 1097–1114

BOOK REVIEWS

Recent Advances in Clinical Neurology No. 6. Edited by Christopher Kennard. 1990. Pp. 276. Edinburgh: Churchill Livingstone. Price £29.95.

The sixth issue of *Recent Advances in Clinical Neurology* contains a balanced appraisal of 9 topics of current interest to neuroscientists and clinical neurologists. The chapters on vertigo, chronic fatigue syndrome, and neurological rehabilitation are valuable current reviews to the clinician. The therapeutic section on epilepsy is a useful overview. The sections on the neural basis of pain, neural transplantation, neurofibromatosis, and cerebral glioma address important growing points in the neurosciences, and the neurology of sphincter control mechanisms is particularly welcome since it has been a too long neglected area of clinical neurological endeavour.

The neural basis of pain by Dostrovsky outlines the physiology of nociceptors, nociceptive neurons and central nervous system pathways with experimental work on the effects of induced injury. The pharmacology of receptor sensitization and transduction is a difficult area, still open to speculation. Nonetheless, the author has carefully reviewed the evidence on substances causing nociceptor activation, sensitization, tissue damage and has extended observations to visceral and cerebral structures. The literature on peripheral and central pain generating mechanisms present helpful and challenging hypotheses which form the basis for much of current research in this field.

Lindvall and Björklund's chapter on neural transplantation in neurodegenerative diseases is essential reading. The particular areas of clinical importance stressed are Parkinson's disease, Huntington's disease and Alzheimer's disease. The experimental animal studies are excellently reviewed, in particular those carried out in rats with hemiparkinsonian-like syndrome, where the functional effects are dependent upon the survival and continuous presence of the graft. Reference to tissues other than adrenal medulla include the sympathetic ganglia, and carotid body glomas cells, suggested as potentially useful in extrapolation to Parkinson's disease patients. The translation of experimental work from animal to human therapeutics is a challenging and compelling story and the authors rightly adopt a critically scientific conservatism. The recent opening up of the transplantation area in the dementias offers an exciting future prospect. These experienced authors conclude that although the limited animal experimental data and early clinical trials in Parkinson's disease are encouraging, this research is still at an experimental stage.

Brandt's analysis of vertigo is a veritable tour de force of the systematic approach to this doughty problem and provides a comprehensive and reliable reference source for the neurologist and neuro-otologist faced with the localization of the origin of vertigo. It covers all the standard clinical syndromes together with the more baffling problems of psychogenic vertigo, and also a well balanced sceptical judgement on the aetiology of disabling positional vertigo.

The chapter by Simon Wessely and P. K. Thomas on the chronic fatigue syndrome is essential reading. This is a particularly careful account of a contentious field. The authors emphasize the variability in presentation of this syndrome, and the importance of accurate definition in a condition which has a number of potential interactive causes. The persistence of viral antigen in a minority of patients may be an epiphenomenon but the authors encourage continued research in this field. They review all risk factors and identify the possible relevance of previous psychiatric illness. They also indicate the need to study the properties of the host in addition to the properties of presumptive causal agents. They advise the need for soundly based epidemiological studies and for a multidisciplinary and open minded approach for the subject. This is an excellently written and referenced chapter.

In neurological rehabilitation, Wade draws attention to this Cinderalla specialty. He aptly comments 'rehabilitation is something undertaken by someone else, somewhere else!'. He rightly stresses the need for neurologists to take an active and enhanced role in the ascertainment, assessment, analysis and treatment of the neurologically disabled and handicapped. Whilst emphasizing the importance of the pathology he shifts the focus to the nature of the handicap and the environment in which this handicap is to be adjusted and treated. He argues for provision of a rational structure against which rehabilitation may be measured, and outlines a practical scientific model for the clinical management in a 'holistic' setting, which would allow the best application of clinical care, with provision for research.

Mathers and Swash address the neurology of sphincter control mechanisms, another important area of distressing handicap in the neurologically disabled and elderly. They convincingly press for a higher profile of interest by the neurologist in this field. The neuroanatomy and physiology of sphincter control are covered faultlessly, with careful background historical review. The integration of somatic and autonomic motor system neural control is imaginatively presented, and the association of specific sphincter dysfunction in clinical neurological syndromes and diseases is invaluable. Investigative tests of the neural integrity of the motor and sensory pathways are elegantly set out by pioneers in this field.

To the clinician familiar with the clinical presentations of neurofibromatosis, Riccardi's chapter comes as a revelation. It is only over the last 20 years, with the explosion of molecular biology, cell biology and genetic engineering that the full implications of the diagnosis of this disease are becoming realized. Riccardi defines the multiple forms of this disorder, indicating that there are at least 7 or 8 subtypes of neurofibromatosis. In addition to classification and clinical descriptions, modern concepts of chromosome aberration, genetic linkage study and mapping techniques provide a quantum move in the understanding of the basic pathology of this disease. From the point of view of future developments in the neurosciences, this chapter opens engrossing new frontiers.

Chadwick's contribution on therapeutic horizons in epilepsy is more easily and immediately comprehensible to the clinician and provides an orderly, rational approach to the neuropharmacology and therapeutic selection, application and trial of anticonvulsants in the control of epilepsy.

The final chapter on cerebral gliomas covers the classification, revised on the basis of experimental tumour models, and addresses the prognosis for treatment and tumour resistance. As in the section on neurofibromatosis, the introduction of newer concepts of molecular and cell biology place this section at the forefront of contemporary understanding of the nature of cerebral gliomas. A logical structure is proposed from which present and future methods of investigation and treatment can be evaluated.

The editor is to be complemented on maintaining the highest standards for *Recent Advances in Clinical Neurology* and for fearlessly choosing areas of difficulty and in some instances neglect. The overall quality of contributions is excellent, and although this is not a book for easy bedtime reading, it is certainly compulsive reading in the quiet of the library or study. It is a granary of accurate and well presented essential information which no aspiring neurologist or neuroscientist can afford to ignore.

Recent Advances in Clinical Neurology is what it should be, a concentrated course of educated scientific essays, successfully designed to move the reader conceptually forward. It is rewarding, enlightening and highly recommended.

J. R. HERON

Neurology. Third revised edition. By Mark Mumenthaler. Translated by Edmund H. Burrows. 1990. Pp. 574. Stuttgart: Thieme. Price DM 44.00.

This is a well written textbook of neurology. The style in translation reads pleasantly, the information is comprehensive and accurate. The text is well indexed, and well referenced.

The author indicates that he understands the limitations of a single authors text in respect of the degree of subspecialization that is taking place within neurology. This proves, however, to be one of the strengths of the book, in that it presents the adapted wisdom of an experienced clinician. The author sets out to present the basic knowledge required for the practice of neurology in a clear, instructive manner and he achieves this with precision and success.

To write a clinical guide to neurology in approximately 500 pages is ambitious and cannot obviously set out to be exhaustive. However, attention to economy of language, the careful choice of excellent tables and illustrations which are relevant and contemporary is a singular and commendable achievement. The classification is sensible and traditional, commencing with brain disease, structured to cover congenital disease, trauma, raised intracranial pressure, infectious and inflammatory disease, and cerebrovascular disease. Systems diseases are considered under extrapyramidal, cerebellar, and metabolic disorder. The spinal cord, nerve roots and peripheral nervous system are dealt with in separate sections, as are the cranial nerves. Important common diseases such as the demyelinating diseases, epilepsy and headache syndromes are considered separately and individually, as are the myopathies and neuromuscular disease.

It would be difficult to seriously fault this book, its limitations are those of any textbook, set against the rapidly expanding fields in the specialty. The author achieves a sound and proper evaluation of the structure of the clinical neurological discipline. It is contemporary, with recent references and free use in the text of authoritative sources, modern classification, a sensitivity to the present state of the art and to the directions and development of the science.

Neurology can be recommended for reference by senior medical students. It is particularly focused for postgraduate doctors training in hospital medicine, and finds its bonding niche in the postgraduate education of neurologists in training. It is a pleasant companion in hospital teaching and also allows neurological registrars or interns a quick, simple and accurate cameo on rarer conditions with immediate access to Fabry's disease, myorrhythmia, nemaline myopathy or Elseberg's syndrome.

This is an enjoyable book to read, and it is refreshing to report favourably on a cogent, coherent and apt flexibook which comfortably represents in reliable outline the basis of European, British and American neurology. The author is to be congratulated.

J. R. Heron

Patient Care in Neurosurgery. Third edition. By N. M. Oyesiku and A. L. Amacher. 1990. Pp. 317. Boston: Little, Brown. No price.

This extensively referenced pocket-sized book is intended for nonspecialists, junior neurosurgeons and nurses in intensive care and neurosurgical units. The authors cover concisely the physiology and perioperative aspects of neurosurgical care and have accomplished their task with commendable brevity and numerous subheadings. Some information is, however, to be found under inappropriate headings: management and statistics of paediatric brain tumours under 'pre-operative assessment' is one example. Several of the line diagrams are superfluous, as are lists of equipment needed for procedures like lumbar puncture. The omission of tracheostomy and artificial ventilation, amongst other topics, can be explained as a reflection of American neurosurgical practice.

Despite my reservations I enjoyed reading this little book and, looking for faults, these are few. It provides a useful guide to ward-based management and explains a little of what takes place in the operating theatre. I will certainly recommend it to senior house officers and nursing staff but feel that there is little to commend it for the neurosurgical trainee.

Colin Schieff

Spinal Cord Compression. By Thomas N. Byrne and Stephen G. Waxman. Volume 33 in the *Contemporary Neurology* series. 1990. Pp. 278. Philadelphia: F. A. Davis. Distributed by Quest-Meridien, Beckenham, Kent, UK. Price £39.72.

As many of us know who work in neurology and neurosurgery, impending spinal cord compression is tragically often misdiagnosed before referral to the appropriate specialty is made. This book written by two American neurologists attempts to communicate to those without specialist knowledge of neurology or neurosurgery a practical approach to the diagnosis of spinal cord compression.

Like many texts dealing with spinal disease a section is devoted to the anatomy and physiology of the vertebral column and spinal cord. There are then two excellent sections, one on the clinical pathophysiology of spinal signs and symptoms and a second dealing with pain arising from the spine which is often the most common presenting complaint of spinal cord compression. There are then sections dealing with nonneoplastic causes of spinal cord compression and a large section devoted to the more common neoplastic causes. The final chapter on noncompressive myelopathy simulating spinal cord compression is very welcome and will be particularly valuable to neurosurgical trainees who will be less familiar with these conditions.

The book places a great deal of emphasis on the diagnosis and investigation of spinal cord compression and to this end there are many excellent reproductions of CT myelograms and MR scans. In many of the sections, however, little space is given to the management and treatment of these conditions and I feel that these sections could have been expanded, particularly for the more common neurosurgical emergencies.

ROBERT BRADFORD

Spinal Tumors in Children and Adolescents. Edited by Ignacio Pascual-Castroviejo. International Review of Childhood Neurology Series. 1990. Pp. 314. New York: Raven Press. Price $130.00.

This is a curious book. It is the sixth volume of the International Review of Child Neurology, sponsored by the International Child Neurology Association. It is written by a paediatric neurologist who has written all but 4 of the 16 chapters. The word tumour should be taken literally, for neoplasms represent only a portion of the lesions described. For example, there are sections on arteriovenous malformations, congenital 'tumors' such as lipomas and dermoid and epidermoid cysts, syringomyelia and even herniated intervertebral discs.

The first chapter deals with the Epidemiology of spinal cord tumors in a very sketchy fashion and is followed by a brief rummage through their pathology. This chapter and the three that follow it (Imaging, Surgical treatment of intramedullary tumors and Nonsurgical management of spinal tumors) are the only ones not contributed by Dr Pascual-Castroviejo. The chapter on the treatment of intramedullary spinal cord tumors, has been supplied by Fred Epstein, who provides, as one would expect, a very lucid account of the presentation, investigation and treatment of these cases. He is particularly interesting on the use of evoked potential monitoring and describes his experience with the Cordis Brain State Analyser, which utilizes optimized digital filtering for averaging potentials which it can detect down to a level as low as 0.10 μV. In his discussion he advocates strongly the surgical resection of spinal cord ependymomas and astrocytomas in children but refrains from describing any long-term follow-up. However, as the most recent of the references that he quotes from is dated 1985, I suspect that the gestation period of this book may have been a lengthy one.

The nonsurgical management of spinal tumours is then described by Drs Curran and D'Angio from Philadelphia, where apparently ependymomas are not to be included as a category of glioma – 'primary gliomas of the spinal cord are more commonly seen than spinal ependymomas in children'. They shrewdly include ependymomas of the conus region and cauda equina in their description of the effects of radiotherapy. These lesions are usually of the myxopapillary type and their sensitivity to radiotherapy makes them excellent material for boosting the efficacy of this treatment in any discussion of the management of spinal cord tumours as a whole.

For the remainder of the book it is back to Dr Pascual-Castroviejo, who deals in turn with everything from neuroblastoma to a slipped disc The general impression from these chapters is that the author has spent a worthy amount of time reading up the literature (the chapter on Tumors of cartilage and bone cites 188 references alone) but that his personal experience of some of the lesions may be limited.

In other words, anyone who requires a literature review on the many subjects dealt with here may well find their $130 well spent (September 1990 prices). But should clinicians be buying a book such as this in order to help them with the practical management of conditions whose incidence is, on the whole, low? Only if the management of such cases is concentrated in units with the appropriate paediatric neurological, neurosurgical and oncological facilities (such as, for example, in Professor Epstein's department) will each individual pathological condition be seen in sufficient numbers to allow a proper experience to accumulate and hence permit the evolution of superior forms of treatment as technological advances in neuroimaging and in medical and surgical care become available.

RICHARD HAYWARD

Head Injury: The Facts. *A Guide for Families and Care-givers.* By Dorothy Gronwall, Philip Wrightson and Peter Waddell. 1990. Pp. 150. Oxford: Oxford University Press. Price £5.95 (paperback).

This book, written by two neuropsychologists and a retired neurosurgeon, is intended for the families of patients who have received a moderate severe head injury. Information in the book is imparted by taking the reader from the patient's arrival in the emergency rooms through typical hospital experiences and illustrating points by using case reports. The majority of the book deals with the neuropsychological sequelae of moderate to severe head injury and I am sure would be extremely useful for those who have to come to terms with a head-injured relative. Though not primarily intended for the clinician, this book would be extremely useful reading for every professional who works with head-injured patients in order that they may better understand the sensitivity and feelings and needs of their patients' families.

ROBERT BRADFORD

Rehabilitation of the Adult and Child with Traumatic Brain Injury. Edition 2. Edited by M. Rosenthal, E. Griffith, M. Bond and J. D. Miller. 1990. Pp. 652. Philadelphia: F. A. Davis. Price £53.23.

This is the second, expanded edition of a textbook which was one of the first in its field. It is aimed at 'all . . . health-care professionals interested in rehabilitating head-injured patients'. Separate sections of the book are devoted to what are termed specific problems; specialized methods of assessment; treatment approaches; and rehabilitation of children. The structure (problems, assessments and treatments) is based on the traditional medical scheme (history, diagnosis and treatment) which is remote from the practice of rehabilitation where, as often as not, the sequence is partly reversed. Most chapters are concerned with individual impairments, and with specific professional activities. This arrangement, again, misrepresents the nature of rehabilitation which is often directed towards problems which transcend professional boundaries. Significantly, there is no chapter devoted specifically to interprofessional collaboration. Most professions involved in rehabilitation are represented among the authors, although nurses are inconspicuous. However, the contributors, or their editors, do not give a convincing demonstration of teamwork, with several substantial examples of reduplication. For example, the management of swallowing disorders is described twice, but yet there is no space for a description of modified diets.

The editors presumably intended most chapters to be used as sources of reference for nonexperts, who need a representative picture rather than a comprehensive one. Some of the contributors give the impression that they are addressing colleagues from within their profession, as though each chapter were a private conversation between experts. It is hard to see how a nonphysician could be interested in a list of 12 routine hormonal tests, especially since the abbreviations are not explained. Lest nonspecialists be overawed, the book is littered with material which is aimed at them, although of little practical value: for example, a table lists the names, but no other details, of 22 tests of learning and memory.

If their purpose is to provide overviews for their colleagues many contributors are guilty of excessive zeal, insisting that deafness in the unconscious patient be assessed with auditory evoked potentials; and describing, either without references or with citations largely culled from nontraumatic cases, conditions such as the locked-in syndrome and the Stevens-Johnson syndrome which have no unique association with brain injury.

An account of visual disorders suggests, with no further comment, that 'if diplopia is causing the deficits in perception, a consultation with the physician regarding modalities designed to reduce the double vision is in order'. In this statement, as in so many like it, weak style belies a deeper emptiness. We are not surprised to read that immobilization of the head and neck 'is inimical to activities that require the patient to move the head'. Banality is especially exasperating when it is shored up with statistics: 'The relatives of the severely injured patients, as might be expected, recorded significantly higher levels of perceived burden ($P < 0.001$) compared with the relatives of the minor injury patients'. Lest this truth be missed it is restated still more vaguely in a later chapter.

Authority should be the raison d'être of authors in a book such as this—otherwise would we not prefer a monograph?—and there is no excuse for describing a so-called 'basal ganglia syndrome' as caused not only by major tranquilizers but also by 'antiseizure and dopaminergic agents, antihistamines, CNS stimulants, lithium, anticholinergics, and oral contraceptives'. It might be better to omit a contentious table purporting to classify 19 drugs according to their liability to cause what are termed 'impaired arousal, impaired movement, epilepsy, (or) impaired cognition'. Similar information is available in pharmacology texts (which do not describe prochlorperazine as an antihistamine); nor would most pharmacologists be so rash as to give a list of 23 'useful drugs with minimal adverse effects'. There are tables without keys, and sentences such as: 'The adaptive value of movement for self-care and -mobility is equally potent for psychosocial skills'. Lapses of this type add to the difficulty of providing a fair review, and reading is made still more laborious by the printers' barbaric word-divisions (for example, on page 298, chosen at random: ma-nipulation; ver-bal; mea-sure; at-tempts). A good book—like good rehabilitation—is much more than its constituent parts. A good multiauthor textbook on rehabilitation should instantiate the principles of goal-setting, structured planning and effective collaboration. This one contains plenty of relevant material, and the inclusion of a section on child rehabilitation is a useful advance on the first edition, but the essence of rehabilitation is not adequately represented.

CHRISTOPHER D. WARD

The Diencephalon and Sleep. Edited by Mauro Mancia and Gabriella Marini. 1990. Pp. 402. New York: Raven Press. Price $72.00.

In an amazing passage from his book, *Man on His Nature*, about the brain awakening from sleep, Sherrington was in no doubt at all that there was much more activity in the awakened than in the sleeping brain. Sherrington, and later Lord Adrian, suggested that a certain level of neuronal activity was necessary for human consciousness. As recently as 1949, Moruzzi and Magoun, from their laboratory at Pisa, viewed sleep as a passive phenomenon, primarily dependent on nonspecific ascending reticular formation mechanisms, despite the previous brilliant clinical and experimental observations from von Economo and Hess. Von Economo, by careful clinical observation of cases in the encephalitis epidemic in Vienna in 1917, had described two patterns of sleep-wake disturbance associated with two distinct localizations of inflammatory lesions in the brain: in cases with excessive sleepiness and ophthalmoplegia, lesions were confined to the posterior part of the third ventricle, while conversely, where insomnia occurred, the lesions were localized to the rostral hypothalamus, tuberal regions or adjacent parts of the striatum. Hess, who shared the Nobel prize with Moniz for his work on leucotomy, presented a film on sleep induced by electrical stimulation of midline thalamic nuclei in a freely-moving unanaesthetized cat in 1927 to the Frankfurt meeting of the German Physiological Society. Since then many coups de théâtre have occurred. One of the most important of these was in 1959 when the reticular theory of sleep was retracted with the recognition that sleep was an active, not passive, process depending on many complex specific systems controlling thermoregulation, autonomic function, the endocrine system and cognitive processes as well as sleep itself. This book is about the numerous and quite fascinating experiments in this field of research since the start of the neurochemical era marked by Jouvet's discovery of the mechanism of rapid eye movement sleep in 1962, and the transfer of primacy in sleep mechanisms away from a nonspecific reticular core to specific brainstem, hypothalamic and thalamic neuronal systems.

There are many problems for the human physiologist or clinician involved with sleep research, not least the lack of primate data with the unsatisfactory task of trying to correlate cat physiology with human experience. Also, a fact emphasized by Steriade in this book, a major problem is raised by the fact that those who possess the electrophysiological tools for the understanding of brain processes ignore the 'wet' data provided by the investigation of chemical sleep factors. Nevertheless, the authors of this book attempt to synthesize these two classes of data in a field which remains dominated by brilliant schools of Italian physiology. A breakdown of authors shows Italy, 28: rest of the world, 36, with contributions from North America 11, Hungary, Spain and Mexico, 5 each, Switzerland and France 3, Netherlands 2 and China 1. The book describes major landmarks in the journey from the reticular formation to the diencephalon, starting with the ascending brainstem projection systems and the complex monitoring of pontine rapid eye movement sleep mechanisms by hypothalamic structures, and ending in the cortex, complex behaviour and dreams.

Bentivoglio, Steriade, Jones, Quattrochi and Hobson give special attention to the projections which ascend from the reticular core and other authors discuss the topic of chemical pontine stimulation. Most of the book is devoted to the hypothalamus and thalamus with major contributions on the role of specific thalamic nuclei and hypothalamic areas. McGinty, Szymusiak, and Parmeggiani stress the interrelationship between thermoregulation and the sleep-wake cycle, with the discovery that insomnia caused by experimental lesions of hypothalamic preoptic areas can be abolished and a normal sleep-wake cycle restored by bringing the ambient temperature to 33° C. Lowering or heightening the temperature can produce an increase or decrease in slow-wave sleep, respectively. Parmeggiani concludes that this area plays a crucial role in the timing of sleep and wakefulness by regulating body temperature. Other results presented by Sterman suggest that the basal forebrain regions are critical for sleep induction, and Sakai emphasizes the role of the posterior hypothalamus in the control of wakefulness.

Several chapters highlight the role of the thalamic reticular nucleus as a pacemaker of EEG synchronization with topographical projections from this nucleus to intralaminar and medial thalamic nuclei. Lugaresi and his colleagues, following their 1986 report of fatal familial insomnia, have now identified 28 subjects with this disorder characterized by dysarthria, ataxia, myoclonus and dystonia as well as by sleep disturbance, accompanied by degeneration of mediodorsal and anterior nuclei of the thalamus. Another chapter of interest to clinicians is that by Krueger who demonstrates that sleep is a cardinal sign of infectious disease and highlights the close relationship between the systems involved in immune response, sleep induction and body temperature regulation with the suggestion that sleepiness in bacterial and viral diseases may be due to the direct action of a cytokine in the brain. Notwithstanding all these stimulating contributions, the exact map of the specific brainstem, thalamic and hypothalamic systems concerned in sleep-wake regulation has yet to be made, and little or nothing is known of the chemical regulation of sleep within the brain in man.

The book is extremely well edited by Mancia and Marini who write the opening and closing summaries as well as contributing to the main text. It is very well produced, illustrated and referenced. There is little or no padding apart from short accounts of polysomnographic investigation in Gilles de la Tourette's syndrome and parkinsonism which make little contribution to the main topic. Not least noteworthy is the frontispiece which shows the authors in front of the striped awnings, geraniums and art deco facade of the Grand Hotel de Iles Borromées on Lake Maggiore. Essential for readers with a strong interest in basic sleep research.

J. D. PARKES

Sleep and Respiration. Edited by F. G. Issa, P. M. Suratt and J. E. Remmers. 1990. Pp. 435. New York: John Wiley. Price $99.95.

This volume, the most recent title in the *Progress in Clinical and Biological Research* series, is made up of 42 papers presented at the First International Symposium on Sleep and Respiration held in Banff, Alberta, in 1989. The study of breathing during sleep appeals to many disciplines ranging from basic scientists and neurologists to respiratory physicians, neurophysiologists, otolaryngologists and radiologists; this is certainly reflected in the authorship of the papers contained in this volume. The conference was partly sponsored by the National Institutes of Health and many of the contributors hold grants from that body.

The book is divided into 10 sections and each paper is followed by a short discussion section. Section 1 deals with aspects of the neurophysiology of sleep and section 2 with the neurophysiology of the upper airway. The former includes an elegant theory by Orem on the nature of the stimulus for breathing during wakefulness. I also enjoyed the paper by Berger and Phillips concerning the relationship between sleep, temperature and energy conservation. Section 3 is made up of a collection of papers dealing with the muscles of the upper airway. Much of the work in this area has dealt with the role of genioglossus and therefore the article by Kuna and colleagues on laryngeal muscle activity during respiration and that by Van Lunteren on the influence of the hyoid muscles on airway patency are particularly interesting and welcome.

Sections 4, 5 and 6 are concerned with upper airway resistance, periodic breathing and other patterns of breathing during sleep. The presentation by Kryger and Hanly looking at patterns of respiration in patients with congestive cardiac failure explains that central apnoea, especially during stages 1 and 2 non-REM sleep, is common and may explain the prolonged survival of these patients if they are given nocturnal oxygen therapy. Section 7 deals with imaging of the upper airway and details ways in which both CT and MRI may be used to assess the airways of patients with obstructive sleep apnoea (OSA) quantitatively and to assess the response to treatment with nasal continuous positive airway pressure (CPAP). Section 8 is devoted to the consequences of sleep apnoea. The papers by Findley on automobile driving and the patient with OSA and that by Roth *et al.* on the mortality of OSA illustrate the seriousness of this under-recognized condition. The mortality of OSA is mainly related to the cardiovascular consequences of the condition and this aspect is dealt with in Section 9. Schmidt-Nowara gives a useful, concise review.

The treatment of OSA includes the use of nasal CPAP and surgery. The final section of this book includes papers which assess these options. Sher's article on surgery stresses the importance of determining the site of obstruction in patients with OSA before a rational decision can be made as to the type of surgery necessary. The final paper by Guilleminault is probably the most useful to clinicians who deal with patients with OSA. He gives sound sensible advice as to how to tackle those patients with mild OSA who do not require CPAP or surgery.

This volume does not aim to educate those uninitiated in the field of sleep and breathing. It is a collection of papers by experts for those already involved in the field. With this caveat in mind, I wholeheartedly recommend it.

N. P. HIRSCH

Neonatal Seizures. Edited by Claude Wasterlain and Paul Vert. 1990. Pp. 336. New York: Raven Press. Price $118.00.

Neonatal Seizures is the published result of a colloquium on pathophysiology and pharmacological management held in 1987. Thus by the time of publication, only 13 of 27 contributions have references to works which are less than 3 years old, and in only 2 of the 27 are there more than three such references. The editors' intention that the text be a useful introduction can still be substantiated since a wide range of aspects are considered, but any serious worker in this field would want more up-to-date information.

There are 5 main sections. That on clinical and electroencephalographic diagnosis is devoted entirely to studies on the human newborn. These highlight the difficulties in deciding on the necessary components of a neonatal seizure. The various authors present differing views and it would have been helpful to have had either a consensus statement or a report on discussion between the participants. The problem of definition pervades the whole of the rest of the clinical aspects of the book, making subsequent contributions in the section on the pharmacology and therapeutics of seizures in the neonate difficult to interpret. Some of these problems are acknowledged by the authors, but their recommendations for therapy remain biased towards old studies in which electroencephalographic monitoring of efficacy was not available.

As might be anticipated, in the sections on mechanisms of brain damage, cerebral blood flow and metabolism, and ontogenesis of receptors and neurotransmitters, most contributions refer almost exclusively to animal work. There is a mixture, which appears to be entirely random, of original studies and review articles. Although the emphasis is present almost constantly, some of the authors have failed to recognize that their subject should be considered in relation to seizures in the newborn, and in some cases the chapters are frankly scrappy. It would have been instructive to have some contributions relating seizures which are symptomatic of inborn errors of metabolism to the biochemical changes which are described in association with seizures per se. The possibility that something useful might be learned from these special circumstances does not appear to have been considered.

Most of the review articles are comprehensive and well constructed, but in a subject in which there has been, in human terms, much recent change in definition and management and, from the experimental aspect, rapid advances in the knowledge of neurotransmission and of seizure mechanisms and their neuronal consequences, a text which relies on a colloquium held 3 years earlier and which costs one United States dollar for every two pages is not a good buy.

SHEILA J WALLACE

Neurobehavioural Aspects of Multiple Sclerosis. Edited by Stephen M. Rao. 1990. Pp. 271. Oxford: Oxford University Press. Price £35.00

I counted no fewer than 8 chapters starting with a reference to Charcot and his observation, made over a century ago, that multiple sclerosis was frequently associated with an abnormal mental state. What is remarkable about this fact is not the perceptiveness of Charcot's observations, but that it has taken over 100 years for such a book as Stephen Rao's to appear. The reason for this delay is frequently alluded to, namely the narrow view held by many clinicians that MS is a disease affecting the nervous system but somehow sparing the psyche, reflecting the uncomfortable feeling experienced by many when dealing with issues such as abnormal emotions and intellectual decline.

Be that as it may, this book is a timely arrival, no doubt stimulated by the upsurge in interest in this area over the past few years. The editor, who has been at the forefront of research into cognitive changes in MS, has divided the book into 4 sections devoted to the clinicopathological features of the disease, cognitive abnormalities, affective disturbance and the management of neurobehavioural dysfunction. The book is for the most part a collection of findings and recommendations already published in the journals although the chapter by Filley *et al.*, reporting some preliminary results from a follow-up study, is new and an important contribution to a virtually neglected area of research, namely the natural history of cognitive change in MS.

It is in the cognitive domain that most recent research activity has been directed and thus a proportionately greater amount of space is given to this area in the book. Disorders of memory, neuroimaging correlates of cognitive decline, the effects of disease course, exacerbations, duration, physical disability and cognitive screening procedures are all thoroughly covered. To those believing in the concept of a specific 'subcortical' dementia, Mahler and Benson's chapter will be welcome although I found their arguments tenuous. As the cognitive bandwagon in MS gathers steam, there is the very real danger that increased knowledge, if incorrectly applied, may do more harm than good. Patients already burdened with an incurable physical disability should not needlessly be exposed to an even greater fear, namely that of intellectual decline, particularly when such decline is not invariable and frequently mild. Therefore, questions such as who and when to refer for psychometric testing and how to place deficits in perspective are of paramount importance. This has been recognized with a fine chapter laying out clear and specific guidelines, which alone warrants a recommendation for the book.

Researchers into neurobehavioural aspects of MS are faced with numerous difficulties which stem in part from the dynamic nature of the disease. As such, findings have often been contradictory. A section on research methodology highlighting the problem areas and suggesting future avenues of exploration would have been useful, perhaps at the expense of a shorter section on clinicopathological aspects, which have after all been well covered in a plethora of books on the subject. If I have another criticism of the cognitive findings, it is the tendency to regard cognition and affect as independent aspects of the mental state. Only in the chapter by Grafman *et al.* is the association between memory deficits and depression explored and one has to wait until the end of the book for Minden and Moes to assert the importance of this relationship.

The section on affective disturbances has contributions by two well-known researchers in the field, Rabins and Schiffer, summarizing their past research on euphoria and affective disorders, respectively. The remaining contribution to this section is a chapter on the association between stress and MS, a particularly murky subject in which cause and effect are difficult to disentangle and which this chapter not surprisingly fails to clarify. Repetition mars the final section containing 3 chapters on management, which nevertheless contains useful information offering a message of potential hope to patients.

In conclusion, this book can be highly recommended, not just to those involved in researching this particular aspect of MS where it provides a useful summary of recent findings, but to all those professionals involved in dealing with multiple sclerosis.

ANTHONY FEINSTEIN

Disturbances of Lower and Higher Visual Capacities Caused by Occipital Lobe Damage. By W. Poppelreuter. Translated by J. Zihl with the assistance of L. Weiskrantz. 1990. Pp. 370. Oxford: Clarendon Press. Price £20.00.

That a monograph written during World War I by a relatively obscure young German neuropsychologist should be selected for translation and republication 75 years later is a remarkable event. It suggests either that the subject has acquired a fresh importance or, as in this case, that the author's ideas were long ahead of his time.

Walther Poppelreuter was in his early thirties when the book was first published; he was trained both in psychology and medicine and was a pupil of Carl Stumpf who did more than anyone to transform psychology from a philosophical discipline to an experimental science. Finding himself in charge of a neurological department for gunshot injuries of the head Poppelreuter resolved to make use of this unique opportunity and conceived the idea of a major four-volume work to incorporate his experience in all types of focal brain wounds including an analysis not only of vision but of speech, sensation and movement. Unfortunately, only the first two volumes were ever published.

Poppelreuter, who originally coined the term clinical psychology, saw it as a clinical department where medical care, psychological therapy, education and vocational retraining could be combined in a way which was not possible in a general hospital setting.

It is inevitable that Poppelreuter should be compared with his British counterpart and contemporary Gordon Holmes. Both worked with similar patients, the survivors of focal brain wounds caused by high-velocity shell fragments, although it has been pointed out that the different design of the British and German steel helmet was responsible for a greater number of occipital injuries in Holmes' patients.

Holmes worked closer to the front line and saw patients at an earlier stage after injury. Poppelreuter's hospital was in Cologne, his patients were studied over a longer period extending for many months after injury and many of the wounds were complicated by the effects of infection or surgery.

However, a more important difference between the two men lies in their approach to the brain-injured patient. Holmes was the quintessential neurologist using clinical observations on patients with focal injuries to deduce the function of localized regions of the brain or of the connecting tracts. The drawback of this approach was the inevitable uncertainty of the anatomical extent of the lesion because of the unpredictable behaviour of high-velocity metallic fragments within the skull, a problem which still beset neurologists such as Ritchie Russell and Spalding studying the same problem 25 years later in World War II. Poppelreuter recognized these limitations and adopted a different approach which was at once more psychological and more pragmatic. He was interested not so much in the site of the injury but in its functional consequences on the patient's ability to communicate, to relate to his surroundings and to relearn his vocation – in short, in rehabilitation.

With this object in mind he devised a series of perceptual tests which he applied to his patients with occipital injuries. In interpreting these he used commonsense quantitative methods such as noting the time to complete a task and was well aware of the importance of variability between patients and the ways in which this could be minimized by using larger numbers. He was also mindful of other pitfalls in psychological assessment – that the results may be markedly influenced by the patient's premorbid intelligence and experience, as well as by global changes, attentional factors and disorders of affect.

It is salutory to reflect, as Poppelreuter methodically develops his theme from lower to higher visual function, how fresh his approach still seems, how well his observations have stood the test of time and how many of his original ideas have been rediscovered in later years.

For instance, he was quick to recognize the limitations of conventional field testing and its variability under different testing conditions. His work on relative hemianopia showing the influence of object size and illumination were the beginnings of quantitative perimetry, to be developed later by Traquaire and by Brodie Hughes. In Poppelreuter's view even a complete and profound homonymous hemainopia could be regarded as a global reduction in performance affecting the whole of the visual field rather than a limited part.

Perhaps because of his lack of interest in anatomical localization Poppelreuter may have underemphasized some important differences in the way visual information is processed by the two hemispheres, notably the predominant role of the right hemisphere in the building up a complete visual percept and in visual memory.

On the other hand, his views on residual vision are sounding echoes to this day in the controversy over 'blindsight'. Poppelreuter was in no doubt that 'being unaware or not reporting a sensation does not mean an absolute absense of sensation' and he also demonstrated the preservation of orienting and averting motor reactions to stimuli in the blind field. His lucid analysis of defects in depth perception, colour perception, localization and attention all of which he found could exist independently in a relatively pure form, has been amply confirmed by later studies. From these observations he deduced that there were multiple connections from the primary visual cortex to the prestriate regions, an arrangement now referred to as parallel processing.

Poppelreuter is no less impressive when he deals with higher visual function in patients with visual agnosia. He realized at once that many patients previously described as having 'mindblindness' had perceptual defects of a simpler kind (as Teuber and Bender were to point out again many years later). He also showed the influence of intelligence and experience on the extent of agnosia.

Poppelreuter's commitment to his patients and to the educational and social implications of his work is obvious on every page; he applied his critical faculties as much to therapy as to other aspects and in his assessments of the various techniques used in rehabilitation he was careful to distinguish the effects of training from the natural processes of recovery.

It is sobering to note that in spite of the extraordinary seminal influence of this monograph on the development of clinical psychology, its author apparently received little recognition in his lifetime. No mention of his name appears in the standard German textbooks of neurology, ophthalmology or psychology and in the English medical literature his fame chiefly rests on his overlapping figures test. Only 6 years after the publication of this book he wrote his last scientific paper although he continued as head of the medical unit at the regional Institute of Clinical Psychology. He died at the age of 53, ironically just before World War II was to generate yet more brain-wounded combatants in need of rehabilitation.

Although it may lack the lucid quality of Gordon Holmes's writing, the book has been clearly and admirably translated by J. Zihl, of the Max Planck Institute for Psychiatry in Munich, assisted by L. Weiskrantz of Oxford.

Oxford University Press and the sponsors are to be congratulated on their choice of this book to be the second in the history of neuroscience series, thereby giving a wider audience the privilege of enjoying an original, important and enduring work of medical scholarship.

R. Ross Russell

The Right Cerebral Hemisphere and Psychiatric Disorders. By John Cutting. 1990. Pp. 479 Oxford: Clarendon Press. Price £45.00.

Although forming part of early 19th century speculation about the brain's duplicity – notably in Wigan's treatise on the subject in 1844 – explanations of psychological illnesses as aberrations of the cerebral hemispheres did not come under full scientific scrutiny until the late 1960s. Revival of the idea then reflected a renewed attempt to establish a biological basis for the psychotic disorders, especially schizophrenia, with the hope, in some quarters at least, that these functional conditions could be redefined as neurological diseases. The latter was not fulfilled, but interest in the biology of mental illness of course continues and an explanation is still required for the undoubted fact that patients with serious psychiatric disorders do deviate markedly on many indices of cerebral lateralization. In this book Cutting offers his own theory as to why that might be. His central thesis is that the symptoms of such disorders arise because of a functional imbalance between the two halves of the brain. The idea is elaborated mainly with respect to schizophrenia where, it is argued, relative underactivity of the right hemisphere, resulting from its pathological dominance by the left, leads to disturbances of perception, thought, emotion, and language involving both sides of the brain. Other conditions more briefly considered – depression, mania, and infantile autism – are explained as variations on this same theme.

Cutting develops his argument progressively through the 3 sections into which the book is divided. Section 1 is a general review of evidence and thinking about the differential functioning of the two hemispheres. Here the author prepares the ground for subsequent presentation of his theory of disorder. He is much influenced by Kosslyn's notion of the left and right hemispheres as control systems (for speech and search), each with a separate sphere of influence but normally cooperative and reliant on the other's competence: it is the disruption of this cooperative state which Cutting later suggests is the hallmark of functional psychosis. The argument that this is associated with a dysfunctional right hemisphere is taken up in Section 2, through detailed consideration of various right focal neurological conditions which have morbid psychological effects reminiscent of those sometimes found in schizophrenia, e.g., disorders of space, of time and of the self and some forms of delusional thought as seen, for instance, in Capgras syndrome. Finally, in Section 3, the author collects together more direct evidence for his theory from research on schizophrenia itself, including studies of the subjective experience and of psychological performance.

In undertaking the task attempted in this book the author has inevitably had to go out on a limb. Like much else about schizophrenia, the data bearing on hemisphere explanations are confused and often contradictory and hemisphere theories, by their very nature, infinitely flexible. Almost any formulation can therefore find some support or be adapted to fit almost any evidence. Cutting's is no exception and, as one progresses through the book, it becomes increasingly difficult to keep track of how theory and fact match up. The reader is not helped by the author's tendency, when in a corner, to become over-dogmatic and dismissive of other points of view. For example, when discussing theories of schizophrenic thought disorder he sweeps away as 'largely futile' the contribution to our understanding made by cognitive psychologists; accompanied by a 60-year-old quote to the effect that 'The discipline of psychology has nothing to say on this matter'. Cutting's complaint here seems to be based on the (wrong) assumption that those interested in the cognitive psychology of thought disorder are not concerned about its neurological basis. It is a pity that he mars an otherwise scholarly discussion with throwaway remarks of this kind.

Nevertheless, this is an unusual and innovative book which should be consulted by anyone looking for a new slant on a topic which is in danger of becoming stale with equivocality. Hemisphere research on schizophrenia has been dominated by, and scarcely progressed beyond, exclusively left-brain theories, justified on the grounds that these are necessary in order to account for the language disturbance perceived as the core feature of the condition. Cutting, more sensitive to the phenomenology of the psychotic experience, challenges such models by reconstruing the impairment of thought and language as an attempt by the left hemisphere to fill in on a fragmented sense of reality which a dysfunctional right brain has helped to create. In doing so he shows how schizophrenia is very much a disorder of the totality of mind, not merely crazy talk. Seen in this light, his hemisphere imbalance theory seems almost as persuasive as Wigan's original version to which, as Cutting himself points out, the thinking behind his own model can be traced. It is a theory whose time may now have come.

GORDON CLARIDGE

Introduction to Neuroimaging. By William W. Orrison. 1989. Pp. 395. London: Churchill Livingstone. Price £43.50.

The decision when writing about imaging is whether to deal with regions or with techniques, and the chapter titles here (in order) suggest some confusion: the spine; the skull; CT and MRI; the head and neck; angiography; myelography; nuclear medicine; ultrasonography; paediatric neuroimaging. Next, balance: 'head and neck' actually includes the sellar region, orbits and temporal bone, but not one of these is allotted more space than the gamma camera and its uses, while a table of the actions and innervation of the extraocular muscles is larger than the text on optic nerve gliomas, which even in an introductory text is questionable.

The text itself is frequently uncritical, relaying radiological lore, and therefore often unintentionally provocative, particularly to be regretted in a book presumably aimed at beginners. Allowance for transatlantic style does not excuse almost invariable poor expression; when doctors assume the role of medical authors, they share the responsibilities of anyone putting professional pen to paper. (The same is true of their editors, who would perhaps be more assiduous if unprotected by anonymity.)

Lastly, the illustrations. Anyone can have the misfortune to have only a poor picture of a rare and interesting case, but here the photographers, printers and art editors must share the blame for a considerable number of substandard figures. The plates of plain films are particularly bad, but as a single example, in fig 3.5 even the numbers indicating the anatomical structures are almost invisible in my copy. I understand that, possibly for cost containment (this book is relatively cheap), publishers in the USA do not send proofs of all figures to the author, and I know of one instance where this resulted in recall and reprinting; Dr Orrison might well make the same case.

IVAN MOSELEY

An Introduction to Magnetic Resonance in Medicine. The basic textbook of the European Workshop on Magnetic Resonance in Medicine. Edited by Peter A. Rinck. 1990. Pp. 130. Stuttgart: Thieme. Price DM 50.00

How much does the practising neuroscientist need to know of the technical aspects of magnetic resonance imaging and spectroscopy? One can argue either way: the basic theory is relatively simple, and the phenomena it describes determine the appearances of the images, so it behoves the clinician to be familiar with them; *or*, beyond the simplest level, the phenomena are so complex (this book lists 30 factors – beside the skill with which the machine is run – controlling image contrast alone) that busy people would be well advised to leave technical details to their specialist colleagues.

I incline to the latter view, not through arrogance, but because I regard these complex details as entirely analogous to the electronics of spectral analysis of the EEG, the methodology of S100 staining or the identification of CSF proteins: merely technical. Neuroscientists who accept that view naturally will find much more than they require in this text, published under the auspices of the self-styled European Workshop on Magnetic Resonance Imaging: 40 pages are devoted to basic principles (the first equation comes on page 3), 45 to technicalities of imaging, 10 each to artefacts and safety; the book ends with a 15 page glossary.

Conversely, this primer will not satisfy the genuinely interested either. It is unclear who has written what, but a thorough revision of the text by a native English speaker appears to have been considered *de trop* by a European Workshop: thus, the book is poorly written, judging by linguistic or didactic criteria (the latter not *invariably* dependent on the former) and in places difficult to follow even with foreknowledge of the subject. It is self-confessedly not clinically relevant, the authors conceding that they 'left the applications in medicine to others'. Despite its modest aims and price, it cannot be recommended.

IVAN MOSELEY

Magnetic Resonance Imaging of the Pediatric Brain: An Anatomical Atlas. By G. Salamon, C. Raynaud, J. Regis and C. Rumeau. 1990. Pp. 364. New York: Raven Press. Price $126.00.

This is the first atlas depicting the anatomy of postnatal development of the supratentorial part of the brain. It is based on MRI imaging of 100 paediatric patients who were considered to have normal studies from which 18 were selected, 11 to cover the period between birth and 18 months and 7, the period between 2 and 16 years. Imaging using predominantly a T_1-weighted anatomy sensitive sequence in sagittal, axial and frontal planes allowed detailed reconstruction of anatomical surfaces. The authors have produced an atlas which beautifully demonstrates the changes which take place during development and present reproductions and line diagrams of midline sagittal, convexity surfaces and a series of axial sections parallel to the canthomeatal line.

The authors emphasize three important features of the developing brain. (1) The constancy of brainstem structure and relationships over this period; (2) the marked changes which take place in the telencephalon; and (3) the orderly progress of maturation of the white matter accompanying myelination.

The atlas will be a useful working reference for assessing actual against expected development at a particular age. It is recommended to departments with a commitment to paediatric neuroradiology and particularly to the study of developmental delay.

BRIAN KENDALL

Pathogenesis and Therapy of Duchenne and Becker Muscle Dystrophy. Edited by Byron A. Kakulas and Frank Mastaglia. 1989. Pp. 288. New York: Raven Press. Price $96.00.

Published proceedings of meetings rarely seem to be of benefit other than to those who have attended the meeting and frequently the preparation of such papers in 'camera ready' style renders the final volume difficult to read. This volume produced by Raven Press from the proceedings of a symposium held in Perth in February 1989 is a notable exception.

The meeting was held to discuss recent advances that had occurred in the field of Xp21.2 myopathies (Duchenne and Becker muscular dystrophy). The participants in the symposium are all acknowledged leaders in their field and simply reading the list of contributors gives rise to a sense of anticipation of what is to be found in the papers and this anticipation is well rewarded. The book is divided into several sections covering: the molecular genetics of Xp21.2 myopathies; pathogenesis of Duchenne dystrophy in the light of the advances of molecular genetics; past therapeutic trials and current corrective procedures. The final two sections contain what appear to be nonedited verbatim reports of the discussions following each section and the final section draws together the whole meeting with recommendations for further therapy.

The format of each section is therefore to have a series of papers following which there is some short discussion and then a more extended discussion at the end of each section. By arranging the book in this way the editors have produced a volume which is of benefit to a wide selection of scientists and clinicians. For someone coming into the field of Xp21.2 myopathies for the first time, either from a clinical or scientific background, the original papers will bring that individual up-to-date as of February 1989. The discussions which follow each individual paper and the round table discussions would be of particular benefit to those who are already involved in the field of neuromuscular disease. By drawing together clinicians and scientists, the scope and limitations of the recent discoveries of the gene and its protein dystrophin are considered.

The editors, Byron Kakulas and Frank Mastaglia, are to be congratulated on producing such a useful volume which should find a place in the personal library of anyone involved in the field of neuromuscular disease and should be in each departmental library. The publishers are to be congratulated in turning the book out so quickly and so free of typographical errors and for producing it at a price which does make it amenable to both personal and departmental purchase.

W. J. K. Cumming

Dynamic Interactions of Myelin Proteins. Edited by G. Hashim and M. Moscarello. *Progress in Clinical and Biological Research*, Volume 336. 1990. Pp. 196. New York and Chichester: Wiley-Liss. Price $59.50.

This volume is from the series entitled Progress in Clinical and Biological Research and contains the proceedings of a symposium on myelin proteins held in Chicago in April 1989.

The book is divided into 6 chapters covering the specialized fields of the authors, each one containing a mix of original data and review material. The opening chapter by P. Morell and colleagues describes the evidence emerging from biophysical studies that myelin is a metabolically dynamic structure which may actively participate in nerve conduction. A further chapter contains an excellent review of pertinent members of the immunoglobulin gene superfamily and their role in glial-axonal interaction and myelination. The authors include numerous carefully designed diagrams showing the molecular organization of these glycoproteins in the myelin membrane. The diagrams are supported by a wealth of morphological and biochemical studies.

The remainder of the book is devoted to studies on myelin basic protein (MBP). There is a thorough review of the posttranslational modifications of MBP and the influence that these may have on MBP interactions in the lipid bilayer. A concise and highly readable account of the shiverer mutant mouse and an MBP transgene of the shiverer describes the relationship between MBP expression and the morphological events of myelination. However, the other 2 chapters on MBP wander from the spirit of the book as suggested by the title. One of these covers the important studies on encephalitogenic MBP specific T cell clones and T cell receptor V genes and goes on to discuss human studies in relationship to multiple sclerosis. The last chapter on antibodies to MBP occupies half the book, almost 100 pages with 243 references. Every angle of measurement and interpretation is reviewed in this scholarly account by Professor Day which leaves the reader in no doubt about his considerable expertise on the subject.

Unfortunately, the book bears all the inevitable hallmarks of similar symposium proceedings: each chapter stands alone as a beacon of academic excellence, being highly informative and well written but totally unrelated to that which it precedes or follows. The chapters have been spliced from a transcript much larger than the MBP gene, thereby creating a group of exons as disparate as the contents of a box of liquorice allsorts. There has been no attempt by the editors to provide a cohesive thread, their preface comprising ten lines of questions. In particular, the marriage of the chapters on myelin protein structure and interaction and the chapters on immune responses to MBP is not a happy one. To be optimistic, however, this relationship may blossom with time. Most of the articles included in this volume are available in review form elsewhere for less than $60. Conclusion? This book is damaging the rain forests.

Hugh J. Willison

Brain Fluids and Metabolism. By Gary A. Rosenberg. 1990. Pp. 207. Oxford and New York: Oxford University Press. Price £30.00.

As the author states in his preface, this 200 page monograph can be loosely divided into three sections. The first 3 chapters deal with the anatomy and physiology of the blood-brain barrier (BBB), cerebrospinal fluid (CSF) formation and circulation, and cerebral metabolism and haemodynamics. The embryonic development of the interfaces between blood, brain, and CSF is outlined in Chapter 2, as is the evidence that the BBB lies at the level of the cerebral endothelium. Chapter 3 is concerned with the relative roles of the choroid plexus and endothelium in forming interstitial fluid (ISF) and CSF, and in maintaining their homeostasis against swings in plasma electrolyte levels. The various mechanisms of passage of substrates across the BBB (active and facilitated transport, free diffusion, and pinocytosis) are discussed.

Chapters 4–6 are concerned about approaches to studying BBB and cerebral function. In Chapter 4 the various ways of measuring blood flow and blood-brain transport of substrates are reviewed. The indicator-dilution and brain uptake index techniques for determining cerebral extractions of test substances are contrasted, as are the use of the Kety-Schmidt, hydrogen clearance, and autoradiographic methods for measuring cerebral blood flow (CBF). In Chapter 5 the techniques of autoradiography and positron emission tomography (PET) and their various applications are discussed, while in Chapter 6 the theory and application of nuclear magnetic resonance spectroscopy (NMRS) is covered.

Chapter 7 contains a more detailed look at glucose, amino acid, and lipid transport and biochemistry in the normal brain. Chapters 8 and 9 are concerned with the pathophysiology and biochemical consequences of anoxia/ischaemia and brain oedema formation, and the mechanisms by which excitotoxic amino acid release, opening of voltage-dependent calcium channels and lipid peroxidation, all contribute to cell necrosis. The results of trials of various therapeutic agents such as calcium channel blockers, steroids, hyperosmolar agents, antiplatelet drugs, and free radical scavengers in anoxia and oedema are reviewed.

This book is extremely well written and easy to understand and the illustrations are excellent. The chapters on the use of nuclear magnetic resonance spectroscopy for studying cerebral metabolism, and on the pathophysiology and treatment of hypoxia-ischaemia and oedema, particularly stand out and can be appreciated in isolation. There are excellent diagrams explaining the theory of NMR, and showing the biochemical evolution of oedema. The initial chapters provide a clear account of the anatomy and physiology of the BBB and CSF circulation, the information being presented in a far more digestible form than that found in specialist works on the BBB.

Chapter 4 details the mathematics of transport of substrates across the BBB and cerebral blood flow determination, and is easy to follow. Surprisingly, although ^{133}xenon washout is still the most commonly used clinical technique for measuring CBF, no section detailing the use and drawbacks of this particular approach was included in this chapter. The description of the techniques of both autoradiography and PET in chapter 5 is also very clear. Examples are provided of the way PET and NMRS can be used to study cortical function and provide prognostic information in cerebral ischaemia, coma, and hydrocephalus, though the potential use of MRI for differentiating cytotoxic and vasogenic oedema is not discussed.

In summary, this book provides an excellent introduction to the subject of cerebral metabolism and haemodynamics, BBB function, and CSF circulation. The book will prove invaluable for any neurologist or physiologist wishing to begin researching in any of the above areas, or for a medical undergraduate who wishes to gain specialist knowledge in this field.

DAVID J. BROOKS

Differentiation and Function of Glial Cells. Edited by Giulio Levi. *Neurology and Neurobiology*, Volume 55. 1990. Pp. 429. New York: Wiley-Liss. Price $102.00.

This volume consists of the proceedings of the symposium 'Differentiation and Function of Glial Cells', a satellite of the 12th Meeting of the International Society for Neurochemistry, held at the Institutio Superiore Di Sanita in Rome during April 1989. Professor Levi is to be congratulated in bringing together 90 scientists, whose names read as a *Who's Who* in the biology of glial cells. The abstracts of the papers presented by the invited speakers make up the text of this latest edition of *Neurology and Neurobiology*.

A wide range of the latest advances in the understanding of the neurobiology of glial cells is covered, under the subheadings of gliogenesis and glial differentiation, hormones and factors (stimulating the division and differentiation of glial cells), cell interactions (between glial cells, neurons and endothelial cells), surface molecules and substrates (including those involved in inhibiting neurite out-growth), electrophysiology and channels (expressed by glial cells), astrocyte functions, myelination and finally glial cells in pathology (of brain oedema, hepatic encephalopathy, demyelination and autoimmune diseases).

One of the clearest messages that came from a number of papers from different sections is that astrocytes can no longer be considered as a homogeneous cell population. Not only do there appear to be astrocytes derived from different cell lineages, but also 'type 1-like' astrocytes derived from different areas of the central nervous system and even apparently homogeneous populations of 'type 1-like' astrocytes may exhibit marked differences in, for example, their electrophysiological and neuropharmacological properties. The paper by Raff is particularly important in this respect, in attempting to define a unifying nomenclature for astrocytes in vitro, so that everyone can at least mean the same thing when they use the same terms. Hopefully future meetings will be able to report the findings obtained by applying these new insights into astrocyte function(s) to the brain in vivo.

One minor criticism of this type of book is the variation in typeface from paper to paper caused by the use of camera-ready abstracts. However, this is offset by the excellent reproduction of the photographs, essential for immunofluorescence and, in particular, the colour plates of astroglial regulation of calcium, which are very striking. The overall standard of presentation of this book is up to that high level of previous *Neurology and Neurobiology* volumes by Wiley-Liss.

The variation in typeface between papers highlights the difference in authors' styles, which vary from excellent to the not so, and reinforces the fact that this book is a collection of abstracts with no overall editorial comment or explanation of the historical background of its subject. This of course is the price that has to be paid for such an up-to-date account of any scientific subject and is meant as no criticism of the editor. As Levi says in his editorial, the book will appeal to those who attended the symposium and even more to those of us (the present author included!) with an interest in the neurobiology of glial cells who were not so fortunate. I would certainly recommend it to such an audience. However, I would not go so far as the publishers and recommend this book to the general clinical neurological and scientific audience, particularly at the price of $102.00.

DAMIAN WREN

Sensory Processing in the Mammalian Brain. Neural Substrates and Experimental Strategies. Edited by Jennifer S. Lund. 1989. Pp. 368. Oxford: Oxford University Press. Price £36.00.

This book is in effect a Festschrift presented by colleagues and students to Dr Gerhardt Werner in the fall of 1986. Werner joined the enormously influential school of Vernon Mountcastle in 1961 at Johns Hopkins. Over the next 25 years Werner led a branch of the Mountcastle school which flourished in Pittsburgh.

It is structured along both palaeo- and neoclassical lines. The older form follows Aristotle's five senses so that there is 1 chapter each on smell and taste, 5 chapters on the body sense, 2 on hearing and 5 on vision. There are 3 chapters on models, the last of which is by Werner himself. The chapters on mainly cortical anatomy and physiology of the sensory systems show examples of the highly professional and precise work which characterizes the group. The final chapter purports to be an announcement of the triumph of Werner's reductionist approach to the senses over what he calls 'the rise and decline of cybernetics'. I take this chapter to be a modern version of pot calling kettle black. One might call it wok slanging the microwave.

The new form of classicism can be summarized as a three-stage process. (1) A subset of cortical neurons transforms incoming information. (2) These subsets interact with other neurons according to defined rules. (3) The operation of these networks constitutes a percept of the sensory world. In other words the neural consequences of a sensed event sweep through a preset sequence of hard wired filters until they activate one which is the unique sign of that event. That is no more than Descartes translated into trendy and takes no account of even Descartes' subtleties let alone Kant and the rest. It ignores the plasticity of the very nerve cells they have so diligently studied. It ignores any possibility of a sensory system actively searching and exploring and questioning by feedback. Their model would provide very rapid images without imagination which is indeed the way some people's brains work.

P. D. WALL

Thalamic Oscillations and Signalling. Edited by Mircea Steriade, Edward G. Jones and Rodolfo R. Llinas. 1990. Pp. 431. New York: John Wiley. Price £89.70.

This, let me state from the start, is a delightful book. It integrates the structure and function of the thalamus in the control of information transfer from the periphery to the cerebral cortex and attempts to provide a theory for the different patterns of activity seen in thalamic cells in relation to various behavioural states of vigilance, i.e., sleep and wakefulness. It represents the 'distillate' of two conferences and subsequent work in this area on (1) thalamic structure and (2) thalamic function in producing oscillatory activity. The structure of the book is very clear. It is organized into 4 parts. First, a historical perspective of some of the observations that have given a key role to the modulation of signal transfer through the thalamus by interthalamic and thalamocortical control systems. Secondly, a very clear exposition of the nuclear, synaptic, inter and intranuclear organization of the thalamic nuclei and their projection to the cerebral cortex as well as the organization of their corticothalamic projections. Additionally, this section shows the paucity of information relating to brainstem-thalamic interrelations. Thirdly, a superb report of the electrophysiology and pharmacology of the thalamic nuclei showing how the connectivity of the thalamic nuclei can lead to oscillatory behaviour of cells. This contains a very clear description of 'voltage-tuned' ion channels and their effects on the discharge patterns of thalamic cells. By this is meant that different ion channels operate at different levels of membrane polarization. Lastly comes the real meat of the book, a report of the experimental evidence showing how the thalamic relay nuclei are functionally switched off during quiet sleep and activated during wakefulness to modulate the transfer of information to the cerebral cortex. These behavioural switches are used to attempt to explain the differences seen in electrocortical activity during sleeping and waking. A large portion of this part of the book relates functional activity in brainstem, thalamic and cortical cells and as mentioned above, the anatomical relationships from brainstem to thalamus are really poorly understood. There is a wealth of information in this book which should be interesting to those who examine, at all levels from undergraduates to postdoctoral, nervous behaviour to understand normal function and those who apply this knowledge to investigate central nervous diseases. It is a pity that the individual chapters do not have concise summaries of their content since this would have made it so much easier to extract, or indicate that the reader had extracted, the relevant messages particularly in relation to overall oscillatory behaviour of the brain seen in quiet sleep. Early on in the book the authors dismiss the theory of thalamic oscillation put forward by Andersen and others in the mid-60s. Careful reading of the book reveals that Andersen's theory provoked a mass of questions and led to much work to elucidate a more acceptable theory from a functional and structural viewpoint. It is a great pity that the authors do not present a résumé of their working hypothesis if only to afford them to be used, in their turn, as a scientific coconut-shy.

ANTHONY ANGEL

ANNOUNCEMENTS

Ninth International Congress of Electromyography and Clinical Neurophysiology

This will be held in Jerusalem, Israel, on June 2–6, 1991. Further information may be obtained from Dr A. N. Gilai, PO Box 29334, Tel Aviv 61292, Israel.

Euromyasthenia III

The Third European Conference on Myasthenia Gravis will be held at Wadham College, Oxford, UK on July 3–5, 1991. Further information may be obtained from Dr A. Vincent, Neurosciences Group, Institute of Molecular Medicine, John Radcliffe Hospital, Headington, Oxford OX3 9DU, UK.

International Quincke Symposium

This symposium, organized under the auspices of the World Federation of Neurology, will be devoted to Barrier Concepts and Cerebrospinal Fluid Analysis. It will be held in Göttingen, on September 19–21, 1991. For information, contact Professor K. Felgenhauer, Neurologische Universitätsklinik, Robert-Koch-Strasse 40, D-3400 Göttingen, FRG.

International Society of Neuroimmunology

The Third International Congress of this Society will be held in Jerusalem, Israel, on October 27–November 1, 1991. Further information may be obtained from the Secretariat, ISNI, PO Box 50006, 61500 Tel Aviv, Israel. Telephone 972 3 654571, Fax 672 3 655674.

International Federation of Multiple Sclerosis Societies

The 1991 Annual Conference and Scientific Symposium of the IFMSS will be held in Amsterdam, The Netherlands, on October 13–17, 1991. Further information may be obtained from Dr C. H. Polman, c/o Free University Hospital, Department of Neurology, PO Box 7057, 1007 MB Amsterdam, The Netherlands.

Second International Congress on Evaluation Scales in Rehabilitation Medicine

This will be held in Montreal, Canada on October 5–6, 1992. The principal themes will be on vertebral dysfunction and head trauma. For further information, please contact Pierrette Boivin, 6300 Darlington Street, Montreal, Quebec, Canada H3S 2J4.

British Sleep Society

The third annual meeting of this Society will be held at Worcester College, Oxford, on September 1–3, 1991. For further information, please contact Dr J. Stradling, Osler Chest Unit, Churchill Hospital, Headington, Oxford OX3 7LJ. Telephone 0865 225236, Fax 0865 225221.

First World Congress of Cellular and Molecular Biology

This will be held in Paris and at the Palais de Congrès, Versailles, France, on September 1–7, 1991. For further information, contact Professor R. J. Wegmann, Residence Haussmann—Bât.A, 1, Avenue du Pavé Neuf, F-93160 Noisy-le-Grand, France.

European Neurological Society

The third meeting will be held at the Palais de Beaulieu, Lausanne, Switzerland on June 27–July 1, 1992. The deadline for the receipt of abstracts is January 1992. Further information may be obtained from Professor Andreas Steck, Service de Neurologie, Centre Hospitalier Universitaire Vaudois, 1011 Lausanne, Switzerland.

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The Guarantors of Brain are prepared to consider applications for part or all of the costs incurred by clinical and nonclinical neuroscientists based in the United Kingdom wishing to visit laboratories or clinical departments at home or abroad. Two schemes exist.

1. Individuals aged under 40 years may apply for the costs of short visits. Applications will be considered for attendance at scientific meetings which relate directly to the applicant's research interests. Only exceptionally will applications to attend larger international conferences be considered. Awards of up to £1000 will be made throughout the year. The Guarantors encourage applicants to meet part of their costs from other sources. Applications should be submitted at least two months before the planned date of departure.

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The following documents are required for awards under each scheme:

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2. an account of the purpose of the visit;
3. the names of two referees;
4. a letter of invitation from the host department;
5. a statement of the costs with details of related applications;
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Four copies should be sent to Professor Alastair Compston, Honorary Secretary and Treasurer to the Guarantors of Brain, Department of Neurology, Addenbrooke's Hospital, Cambridge, CB2 2QQ (Tel. 0223 217091).

All successful applicants are expected to submit a short report of the visit on their return.

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